COLLEGE of AMERICAN PATHOLOGISTS

Applying the CAP-ASCCP Lower Anogenital Squamous Terminology (LAST) Project Principles in Clinical Practice:

Case Examples Illustrating Biomarker Usage

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Disclosure

- **Dr Ronnett served as an expert panel member for the Intraepithelial Lesions Work Group (WG2) of the CAP-ASCCP LAST Project.**

Interest/Activity Type	Entity	Year Submitted
Consultancy	Merck Research Laboratories	2012
Lectures Fees paid by Entity	MTM Laboratories	2012
Grants	NIH/NCI	2012
Grants	Merck Research Laboratories	2012
Royalties	Blaustein's Pathology of the Female Genital Tract (Springer Verlag)	2012

Topics/Objectives

- **Discuss classification of cervical squamous intraepithelial lesions**
- **Explain rationale for use of certain biomarkers to evaluate HPV-related cervical lesions**
- **Illustrate biomarker expression patterns and how to incorporate results into final interpretation/diagnosis**

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Classification of Cervical Squamous Intraepithelial Lesions

Mild dysplasia	Moderate dysplasia	Severe dysplasia	Carcinoma in situ
CIN 1	CIN 2	CIN 3	CIS

CIN = cervical intraepithelial neoplasia

LSIL (CIN 1)	HSIL (CIN 2/CIN 3/CIS)

SIL = squamous intraepithelial lesion (low-grade and high-grade)

LSIL/CIN 1 = transient HPV infection
(oncogenic [~85%] and non-oncogenic [~15%] HPVs)

HSIL/CIN 2 = mix of precancerous lesions and LSIL/CIN 1

HSIL/CIN 3/CIS = precancerous high-risk HPV-related lesion

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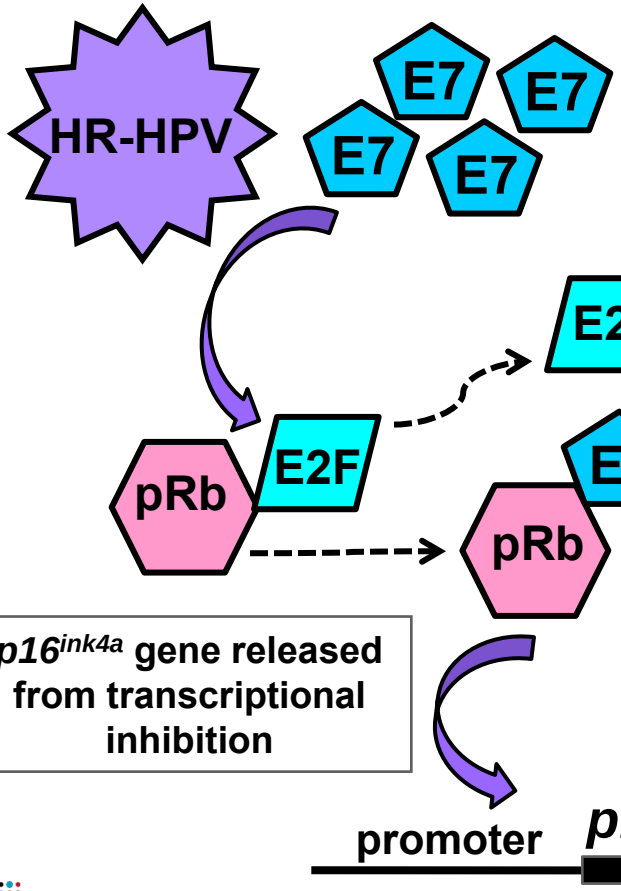
Rationale for Improving Cervical Biopsy Diagnoses

- **Cervical biopsy diagnoses determine treatment**
- **2 goals of biopsy interpretation:**
  - **Diagnose dysplasia (SIL/CIN) versus normal**
  - **Distinguish transient lesions (LSIL/CIN 1) from precancerous lesions (HSIL/CIN 3, some CIN 2)**
- **Diagnosis of cervical lesions on H&E-stained sections is affected by interobserver variability**
  - **CIN 2 is the least reproducible category yet serves as the treatment threshold**
  - **Misclassification of normal as CIN 1 is common**
  - **HSILs can be misclassified as negative when small, fragmented, or altered by reactive/metaplastic changes**

High-risk HPV-mediated Disruption of Cellular Mechanisms via Deregulated HPV Oncoprotein Expression Results in p16 Over-expression and Proliferation

Productive infection → Transforming infection*



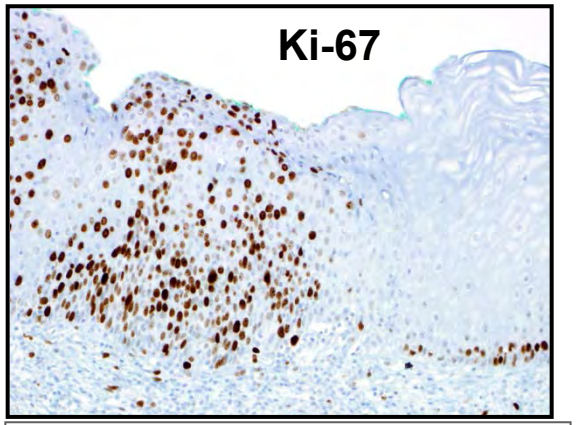
E7 over-expression

Activation of CDKs; transcriptional activation

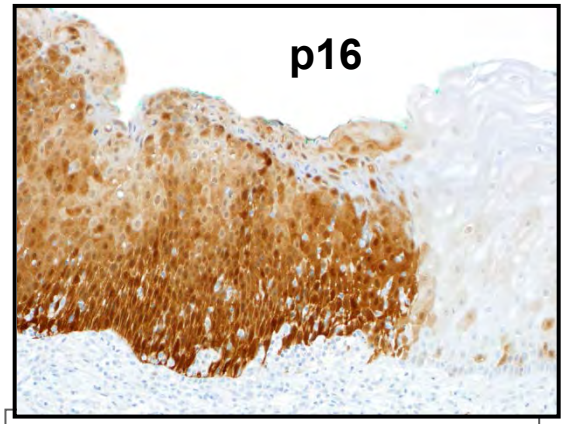
E7 inactivates pRb

***p16ink4a* gene released from transcriptional inhibition**

p16 (CDK inhibitor) attempts to control cell cycle



Cell cycle progression (↑proliferation)



p16 over-expression

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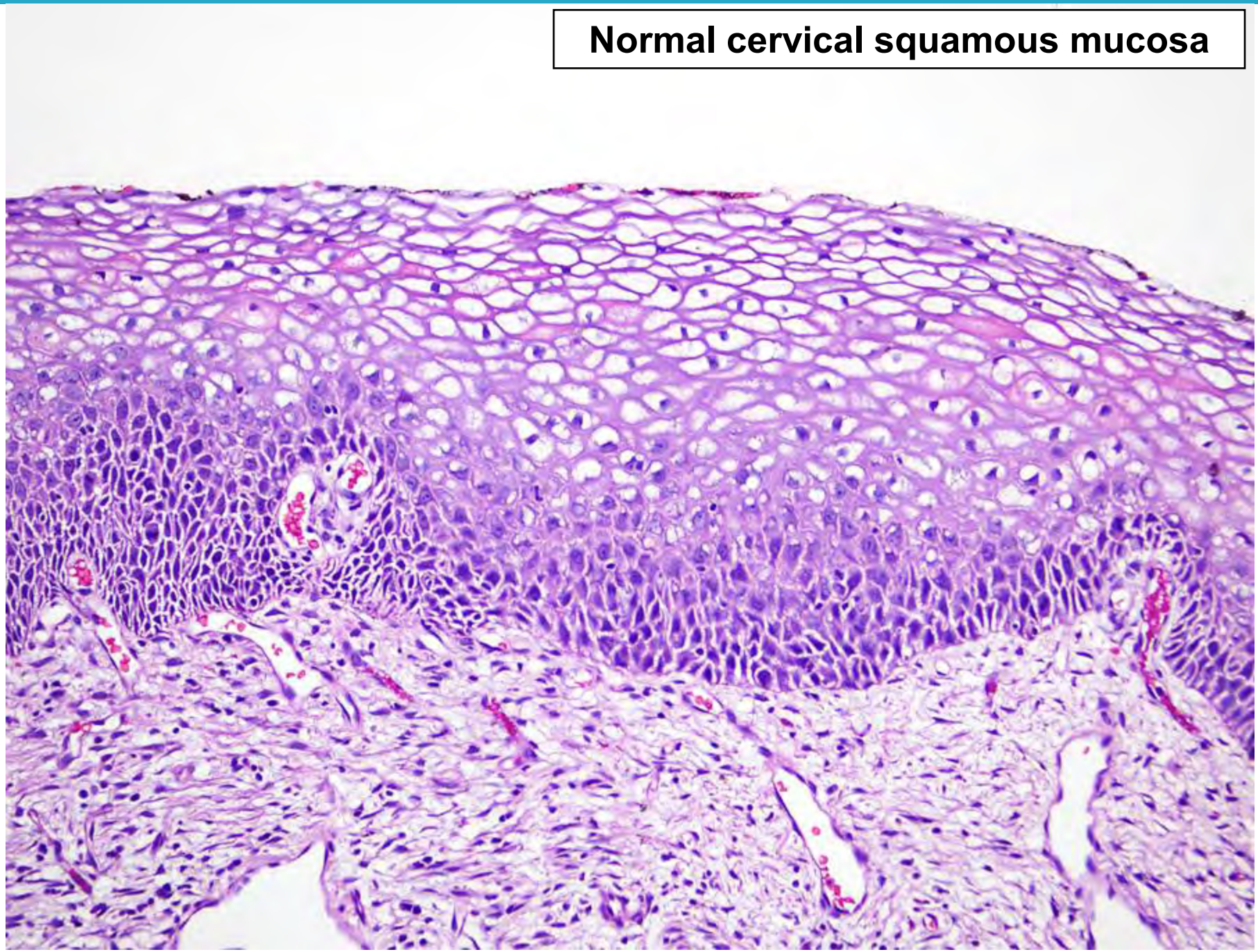
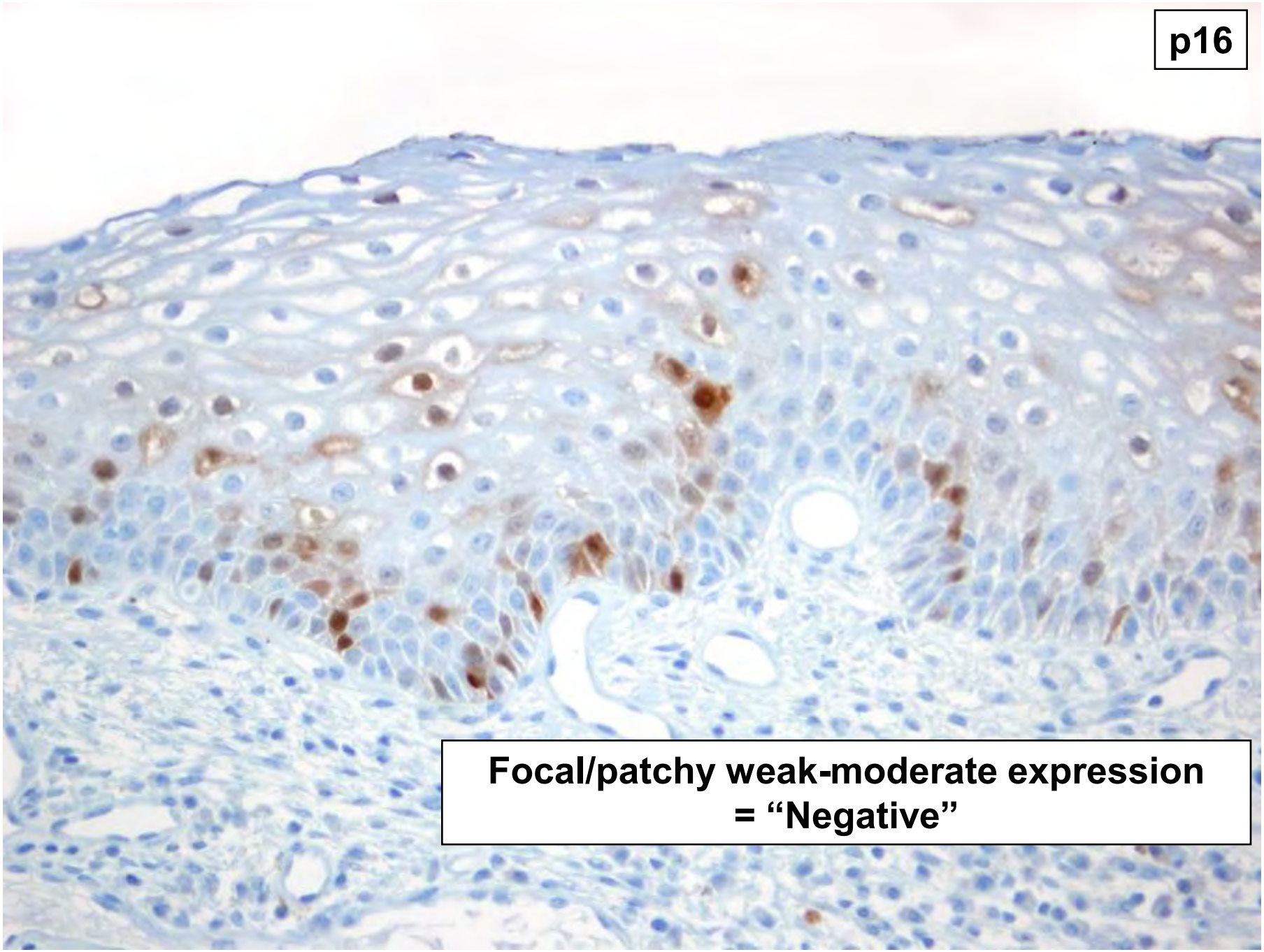
***also involves E6 over-expression and p53 inactivation**

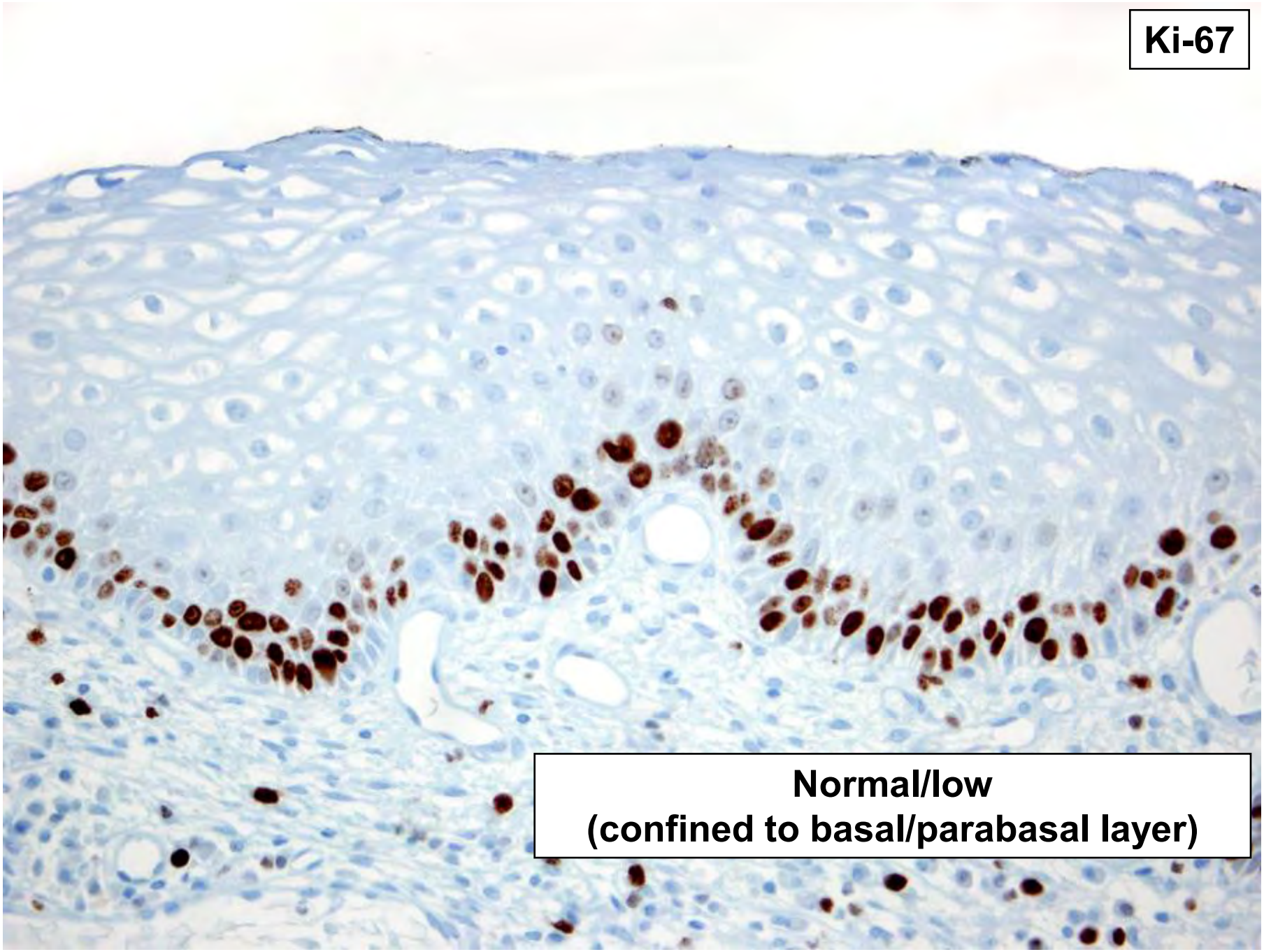
Cervical Intraepithelial Lesions: Biomarker Patterns

Coordinate expression patterns	Ki-67 ↑	Ki-67 normal/low
p16 + (diffuse/strong)	High-risk HPV-related intraepithelial lesion	?
p16 –/f+ (negative or focal/patchy)	?	NIL

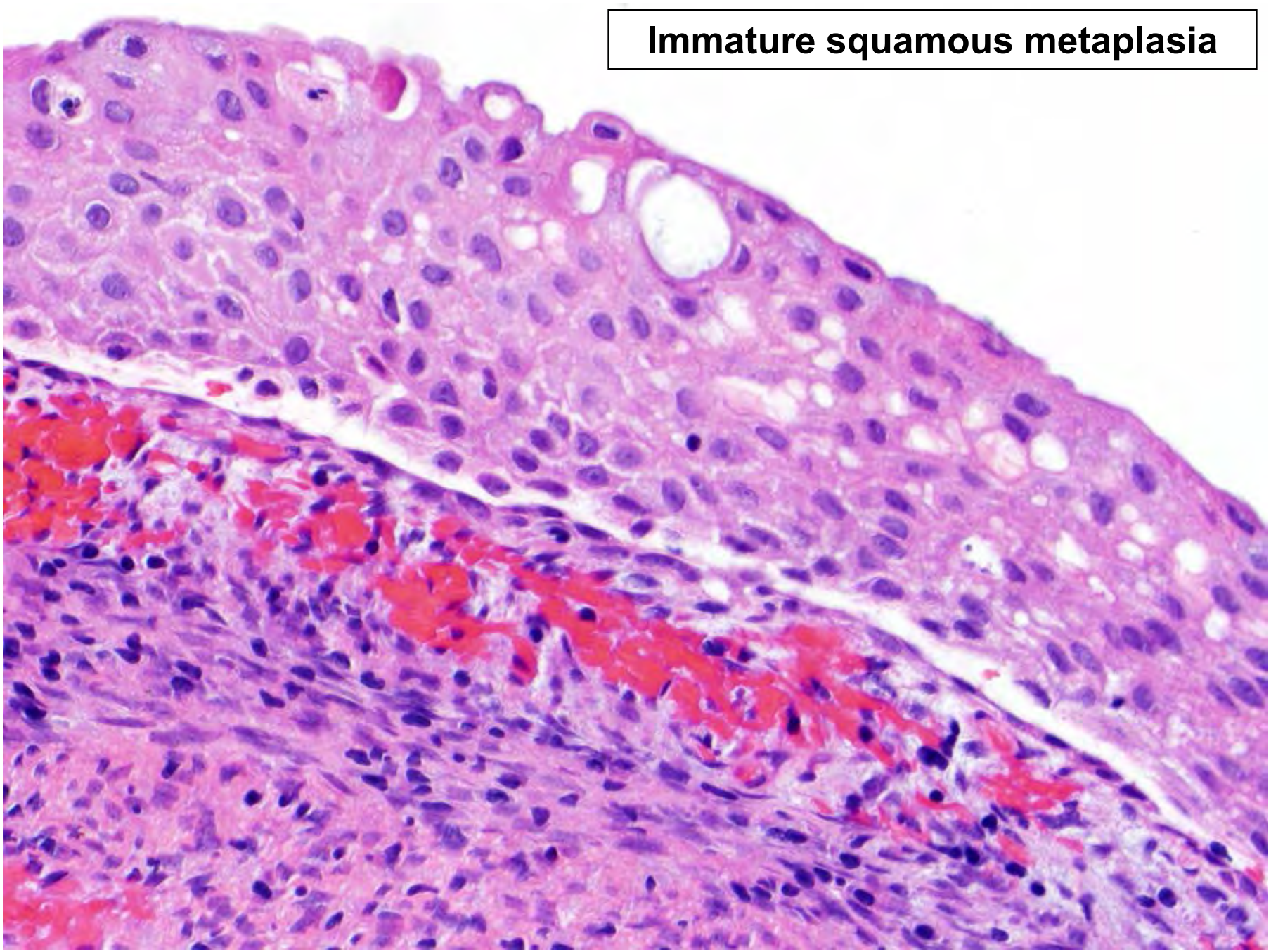
Normal cervical squamous mucosa

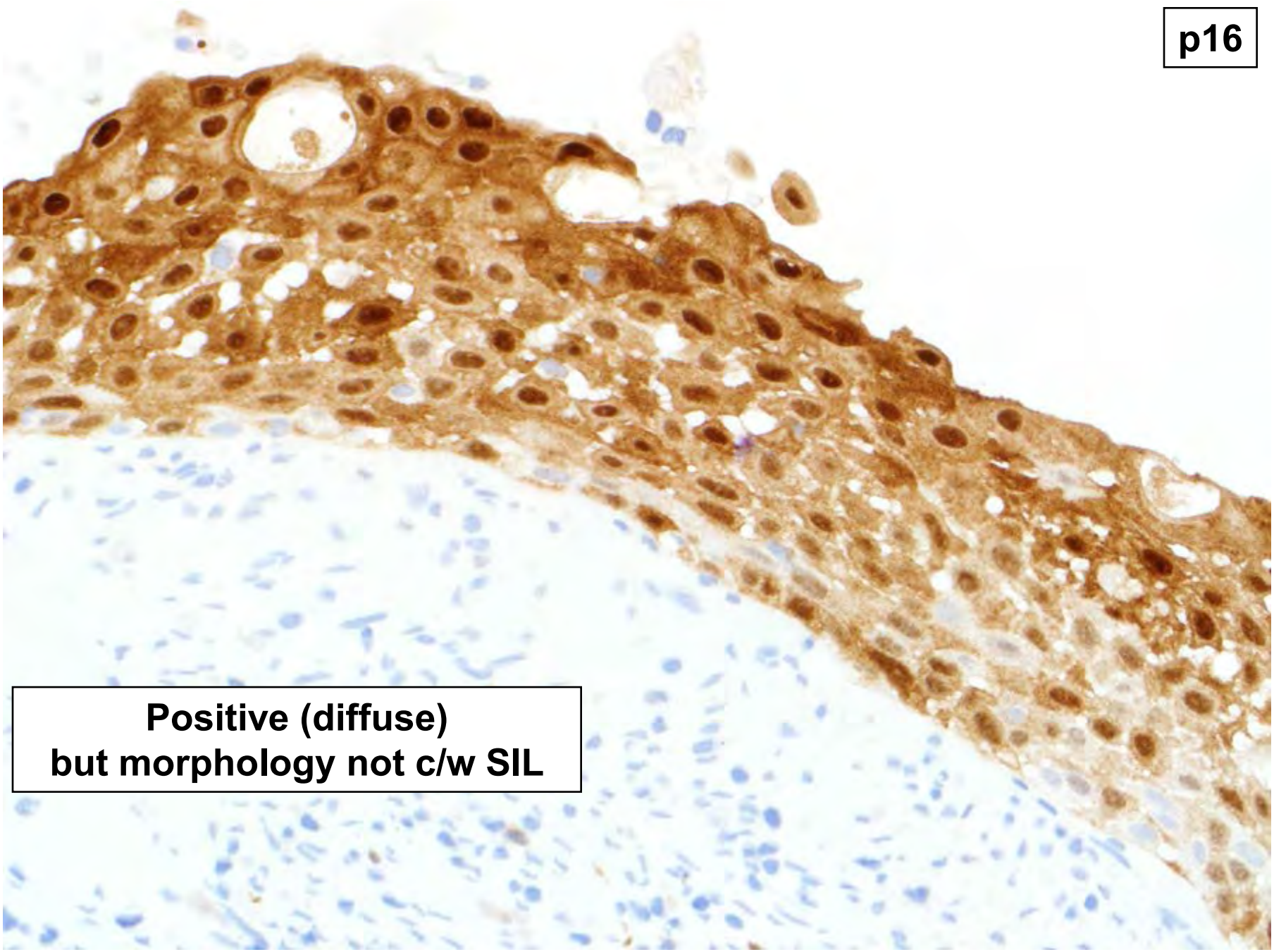



p16
Focal/patchy weak-moderate expression
= “Negative”

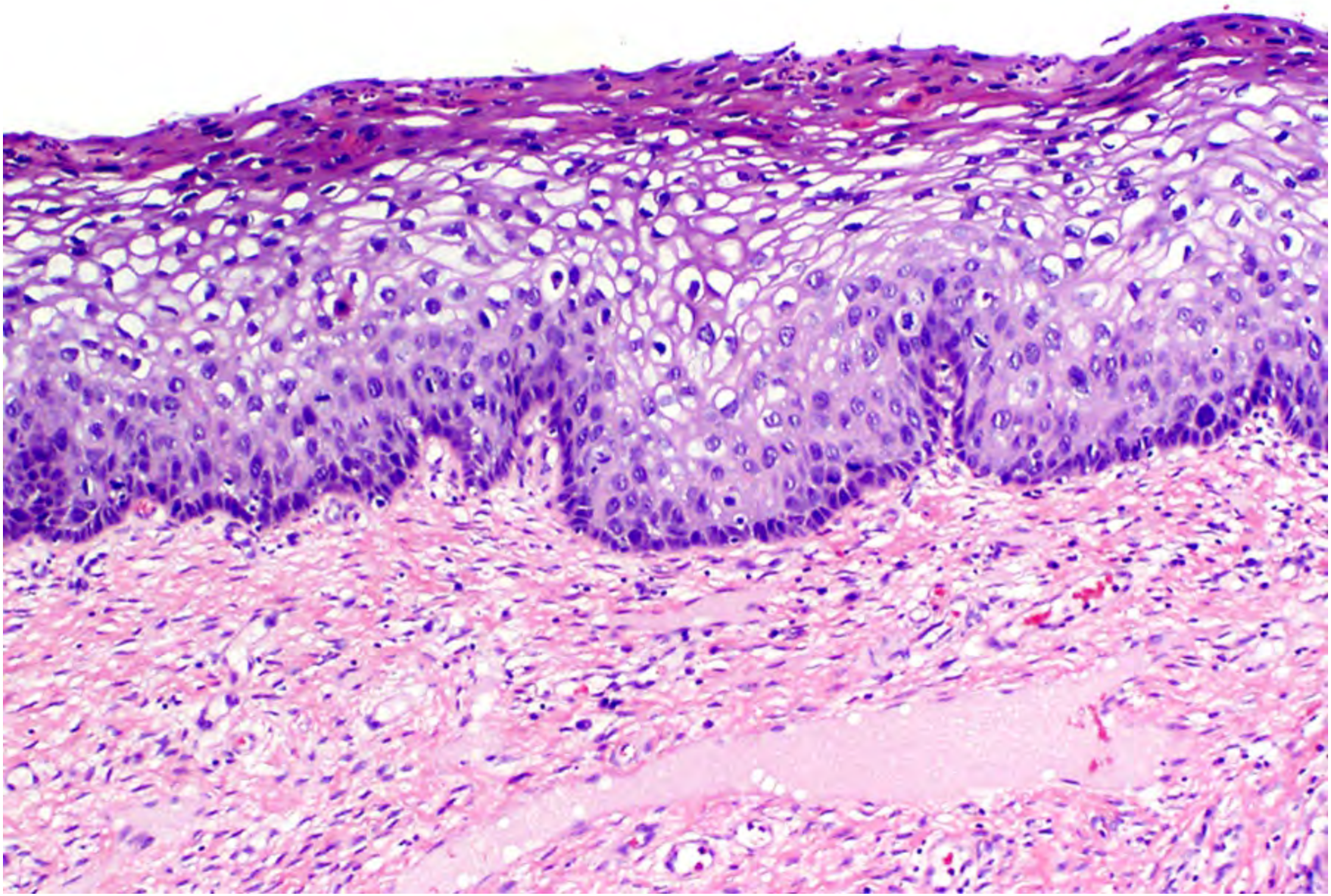

Ki-67
Normal/low
(confined to basal/parabasal layer)

Immature squamous metaplasia



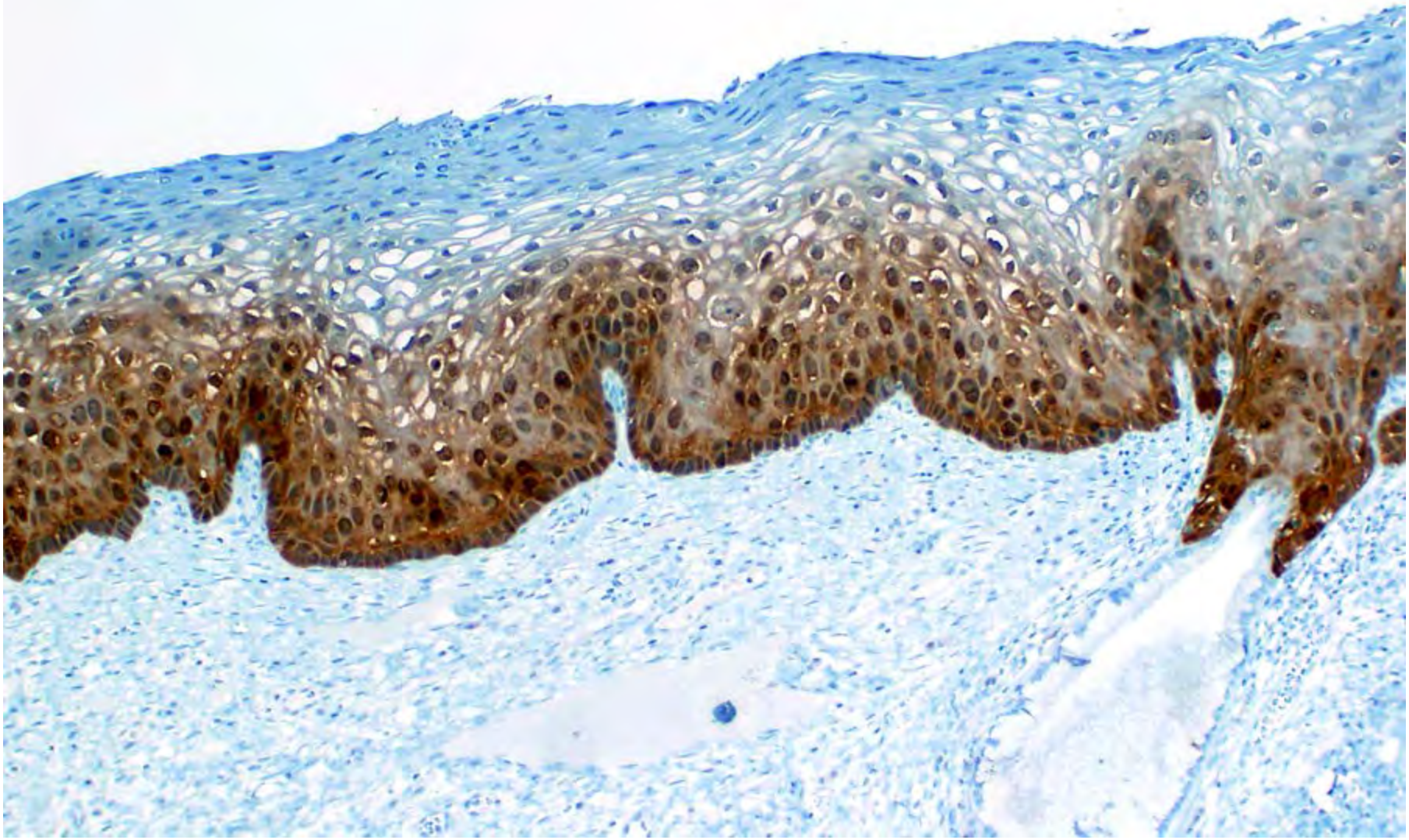

p16
Positive (diffuse)
but morphology not c/w SIL

LSIL/CIN 1



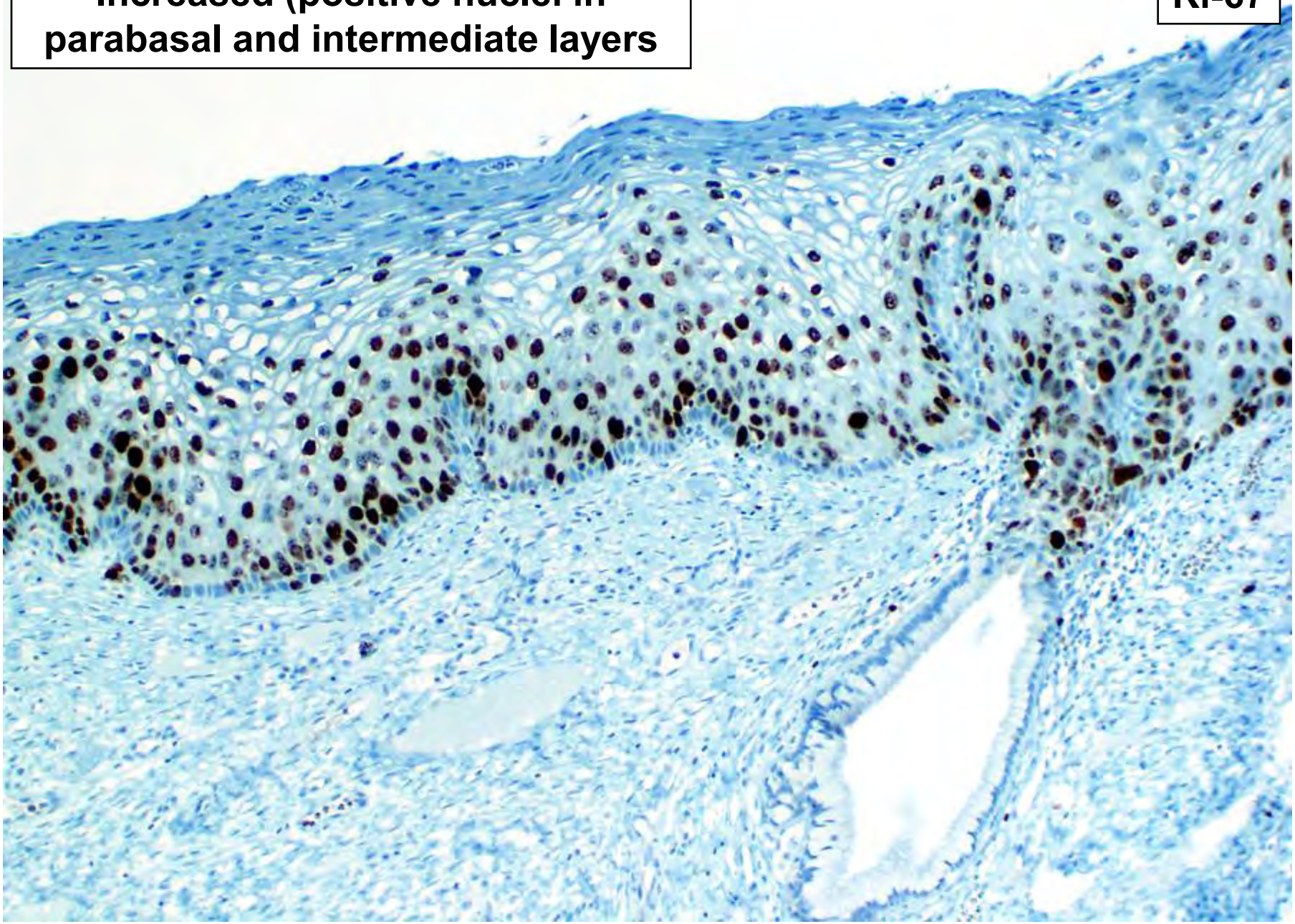
Positive = diffuse/strong
(continuous along basal/parabasal)

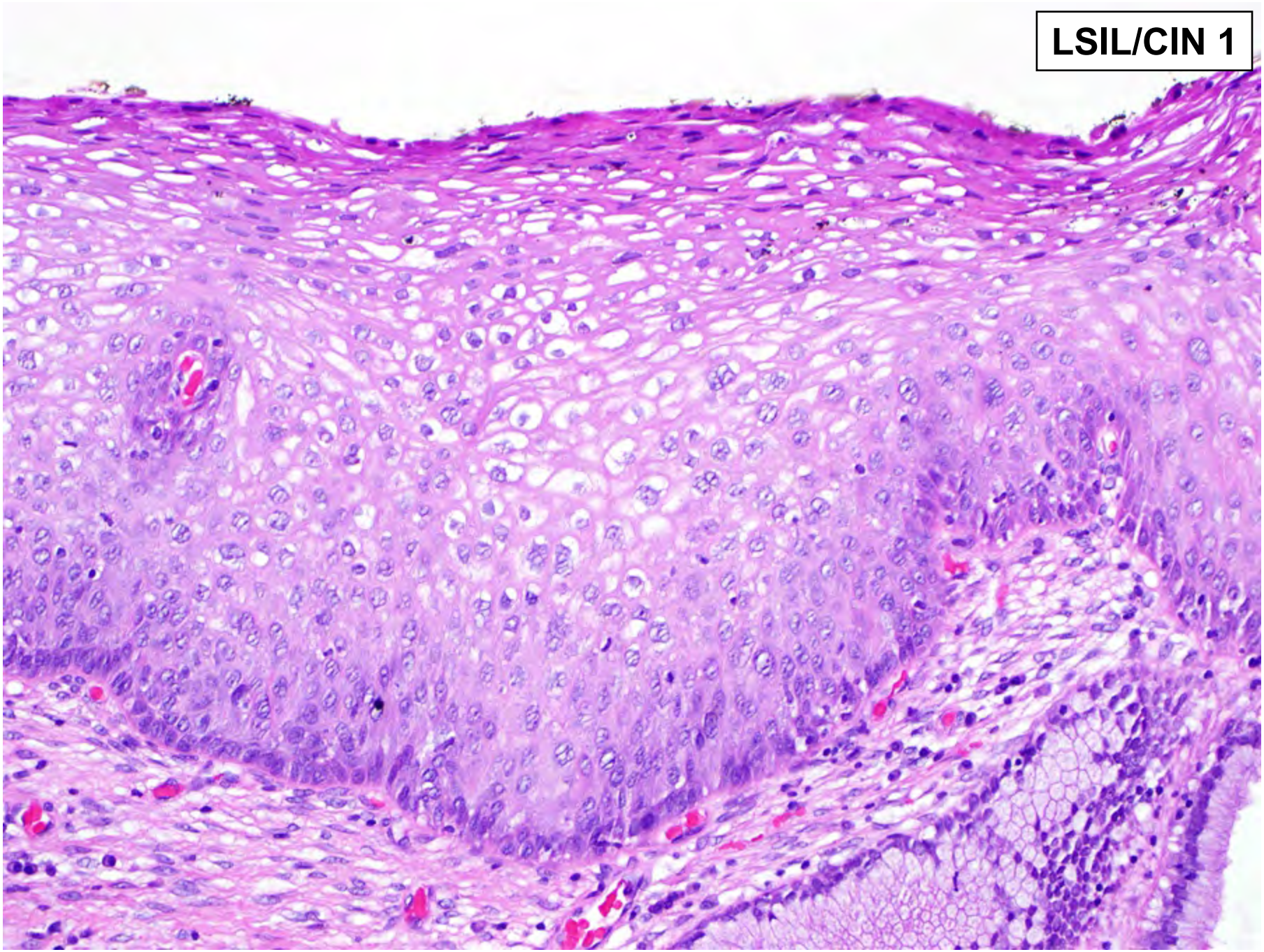
p16

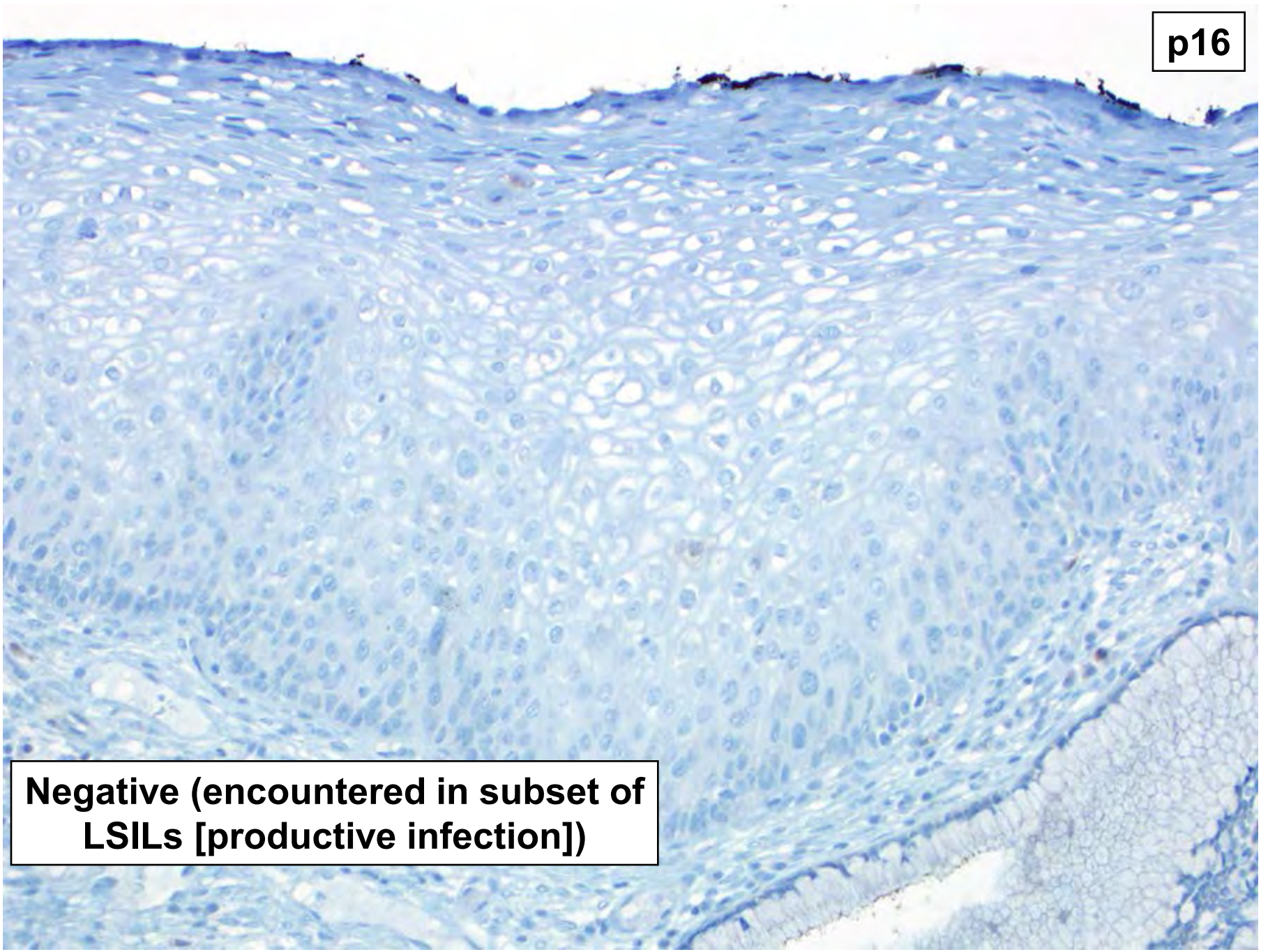


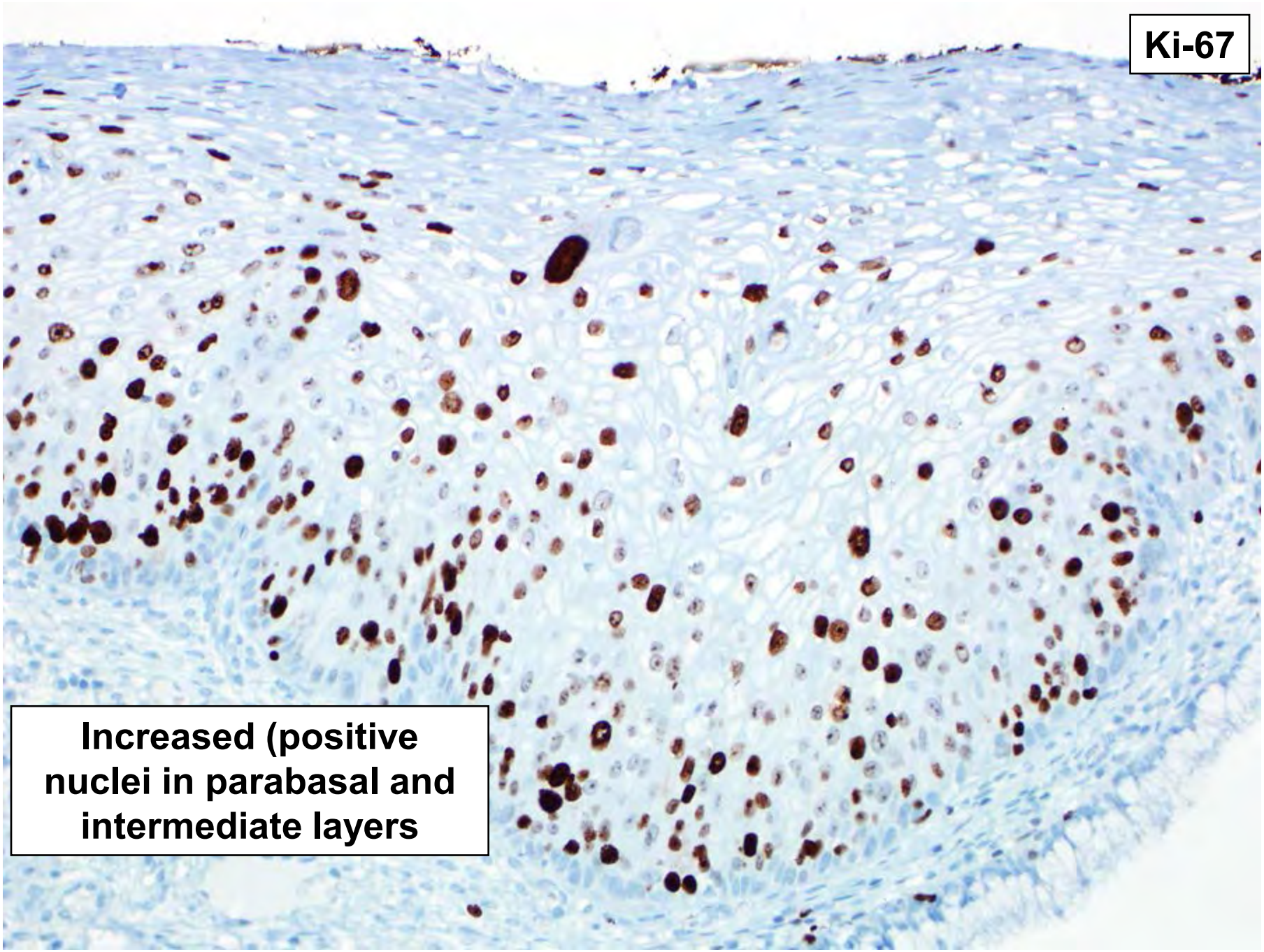
Increased (positive nuclei in parabasal and intermediate layers

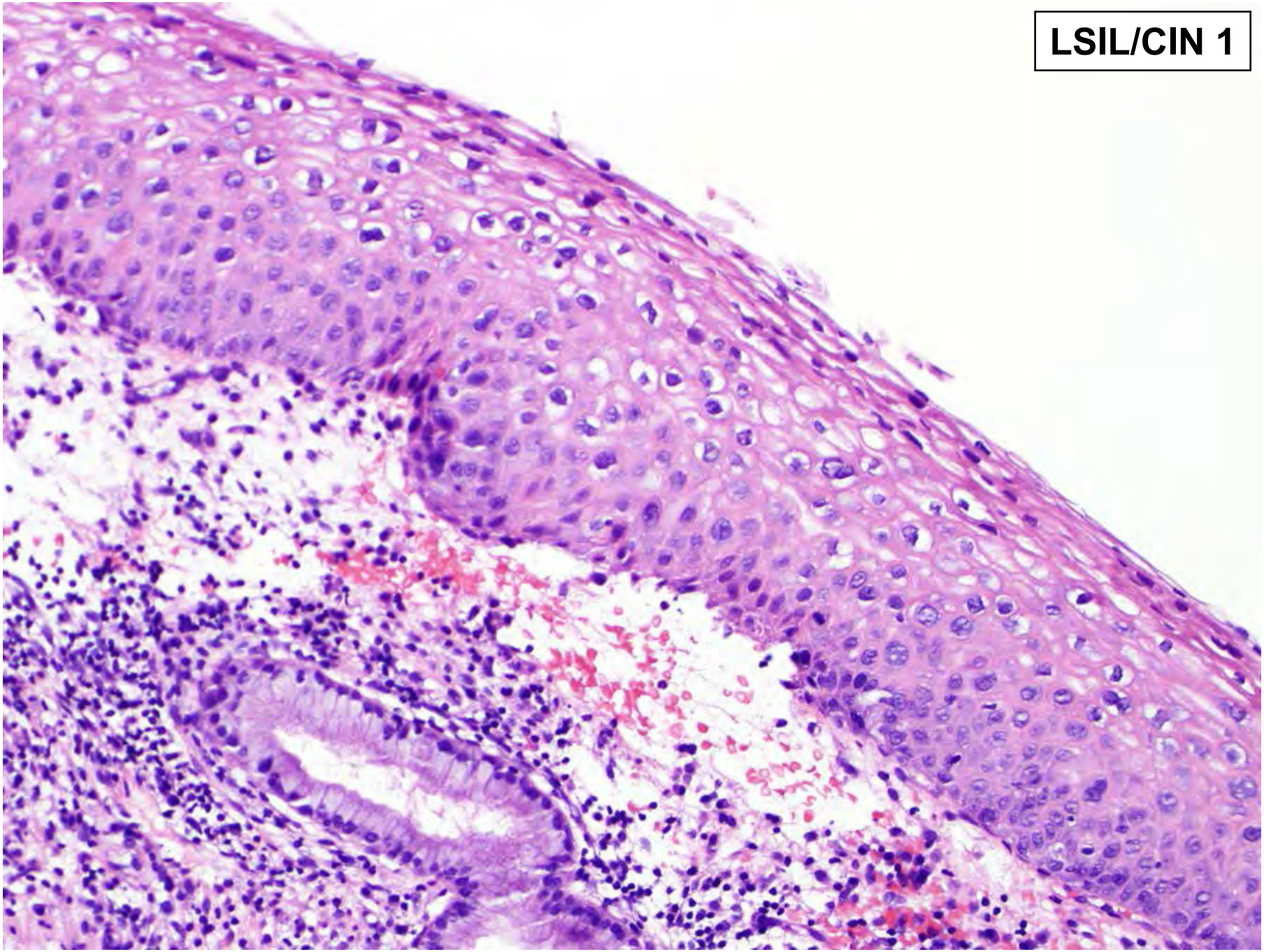
Ki-67

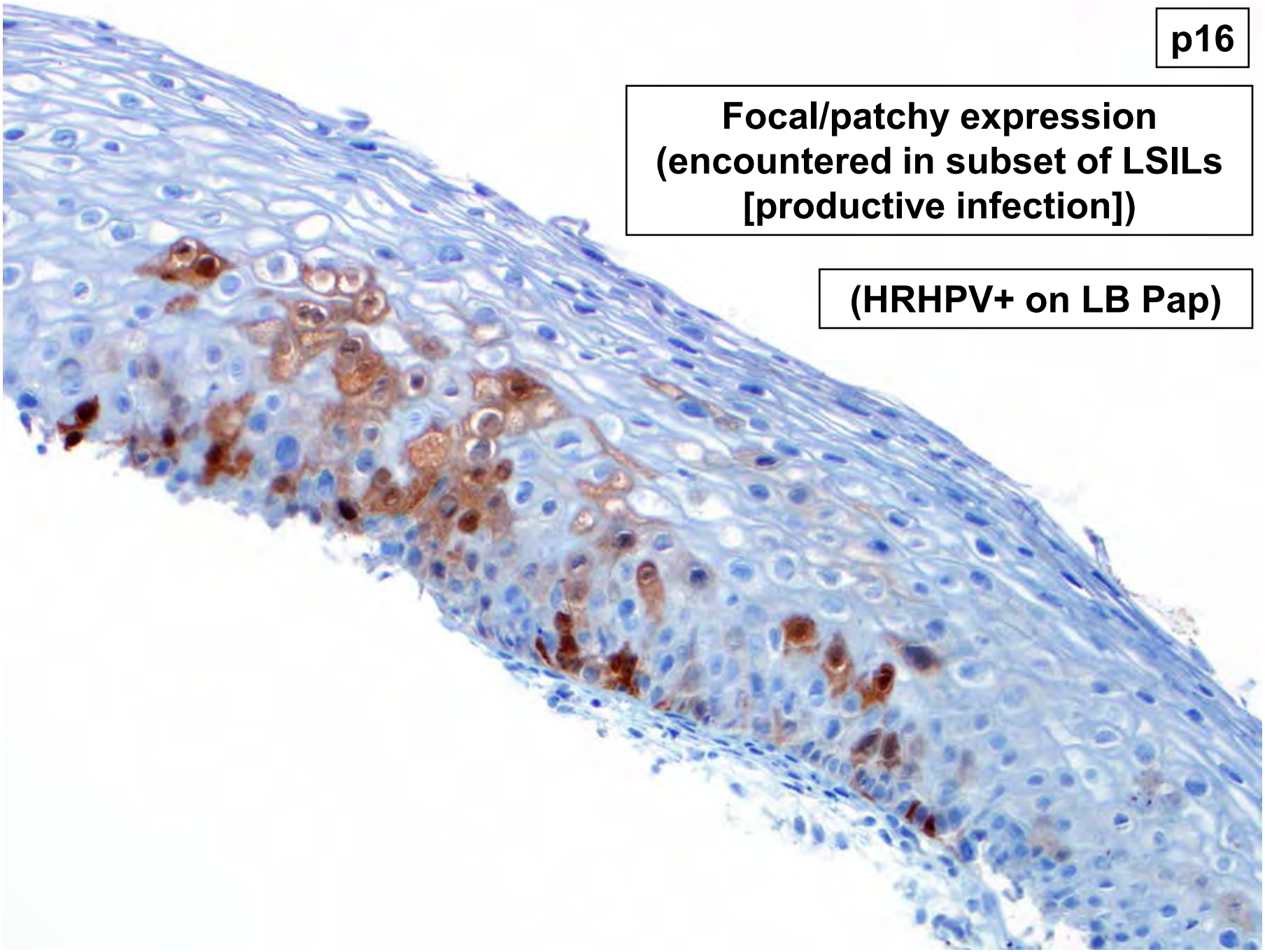


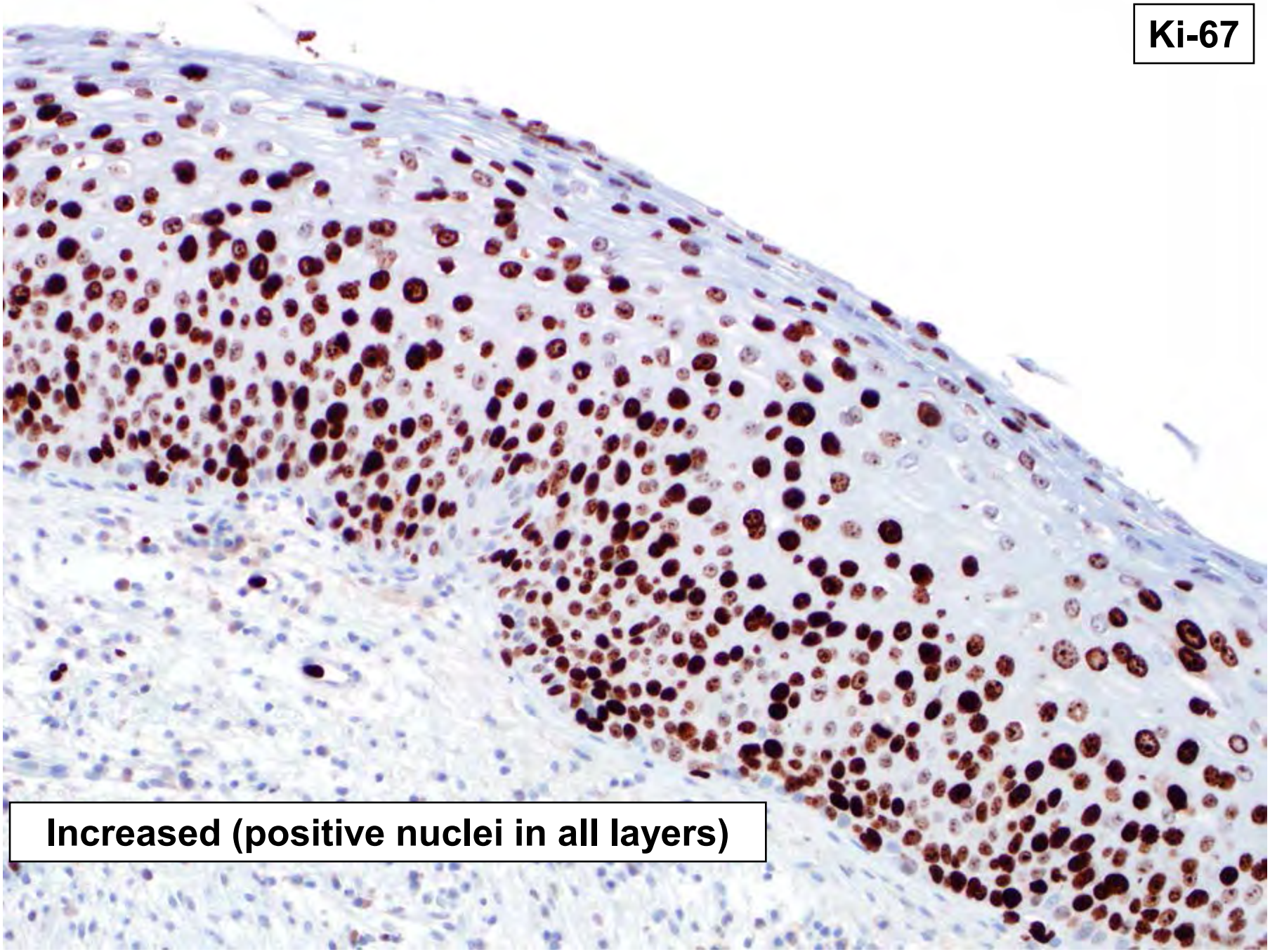

LSIL/CIN 1

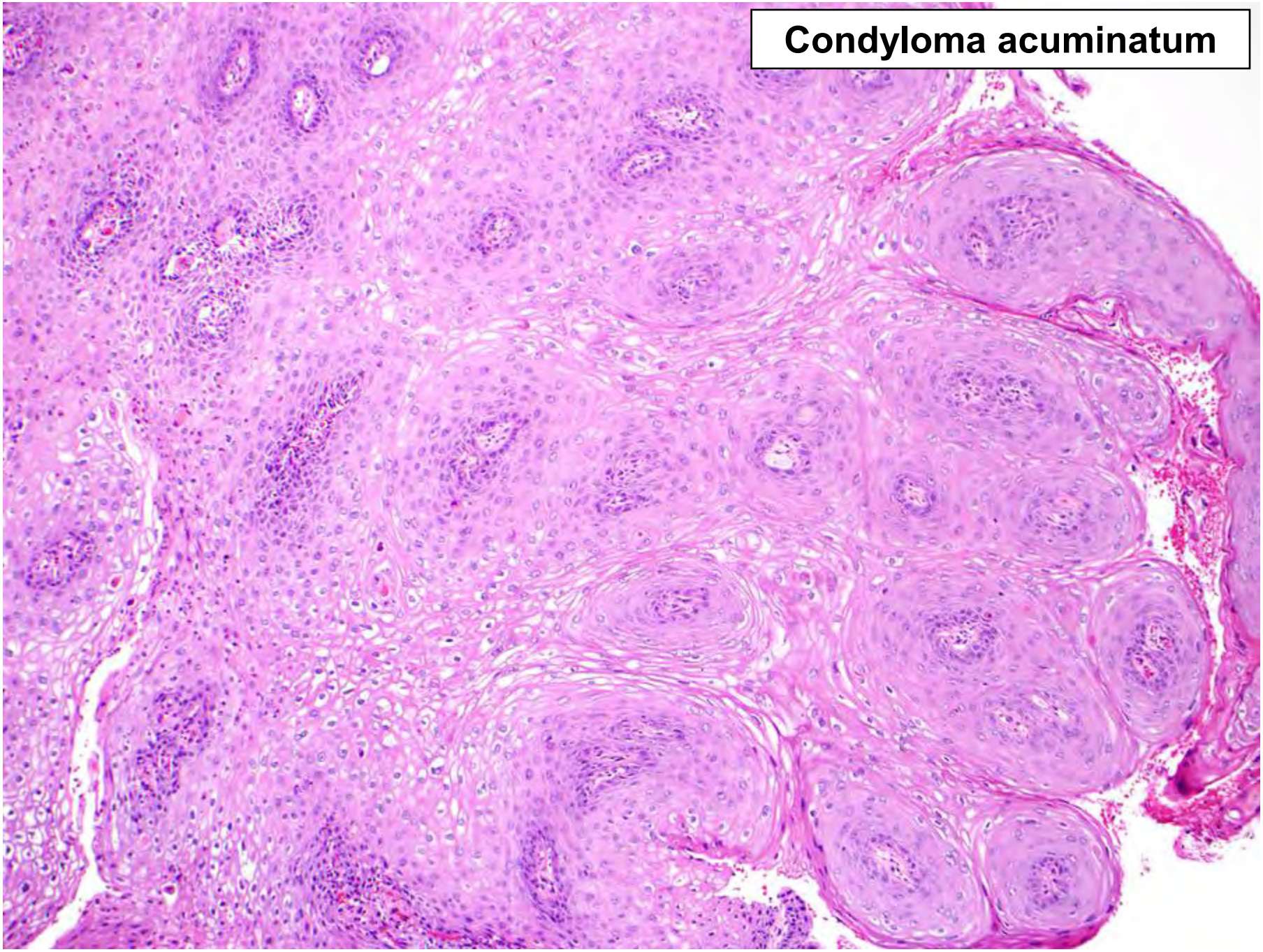

p16
Negative (encountered in subset of LSILs [productive infection])


Ki-67
Increased (positive nuclei in parabasal and intermediate layers


LSIL/CIN 1

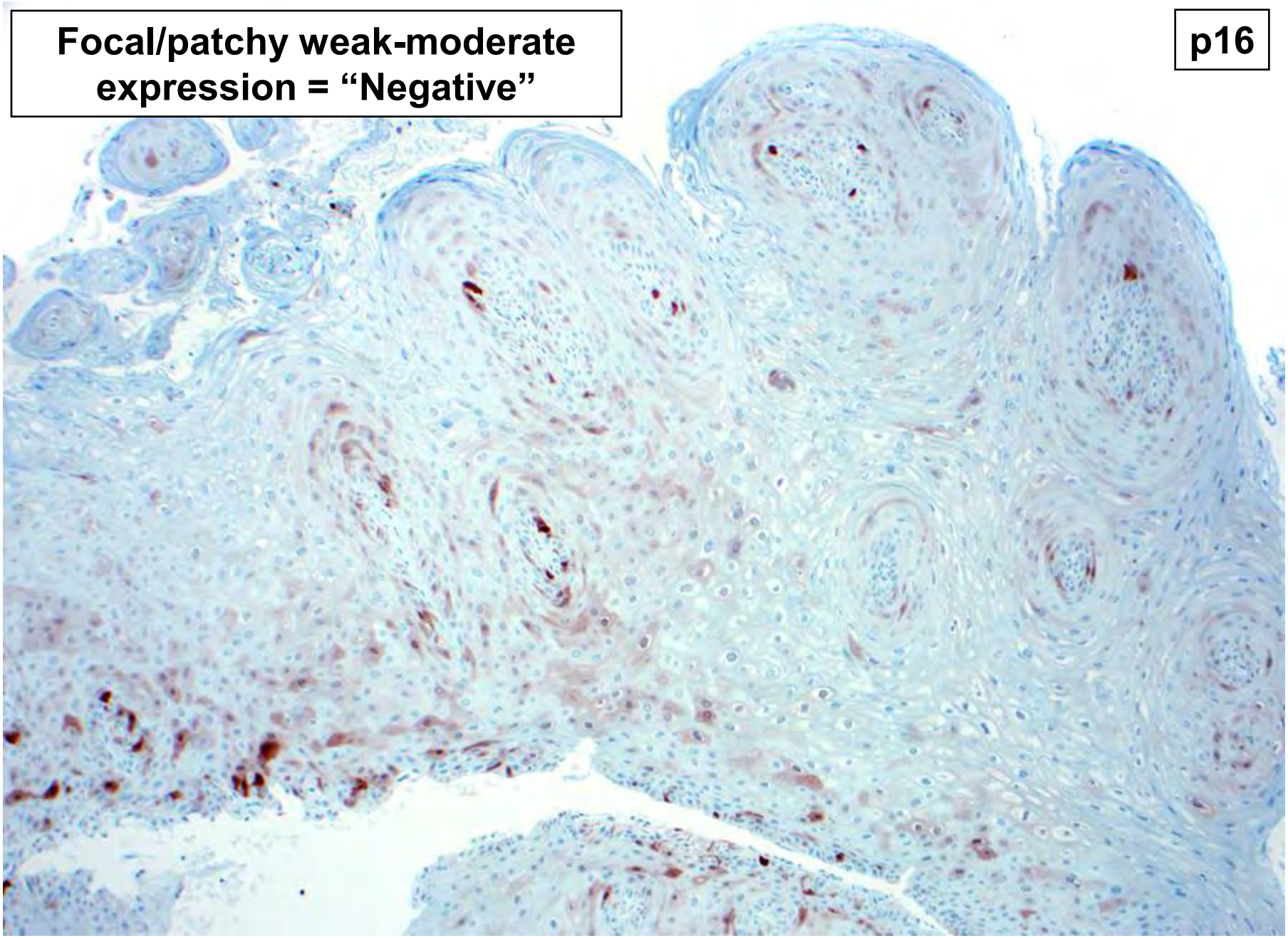

p16
Focal/patchy expression
(encountered in subset of LSILs
[productive infection])
(HRHPV+ on LB Pap)


Ki-67
Increased (positive nuclei in all layers)


Condyloma acuminatum

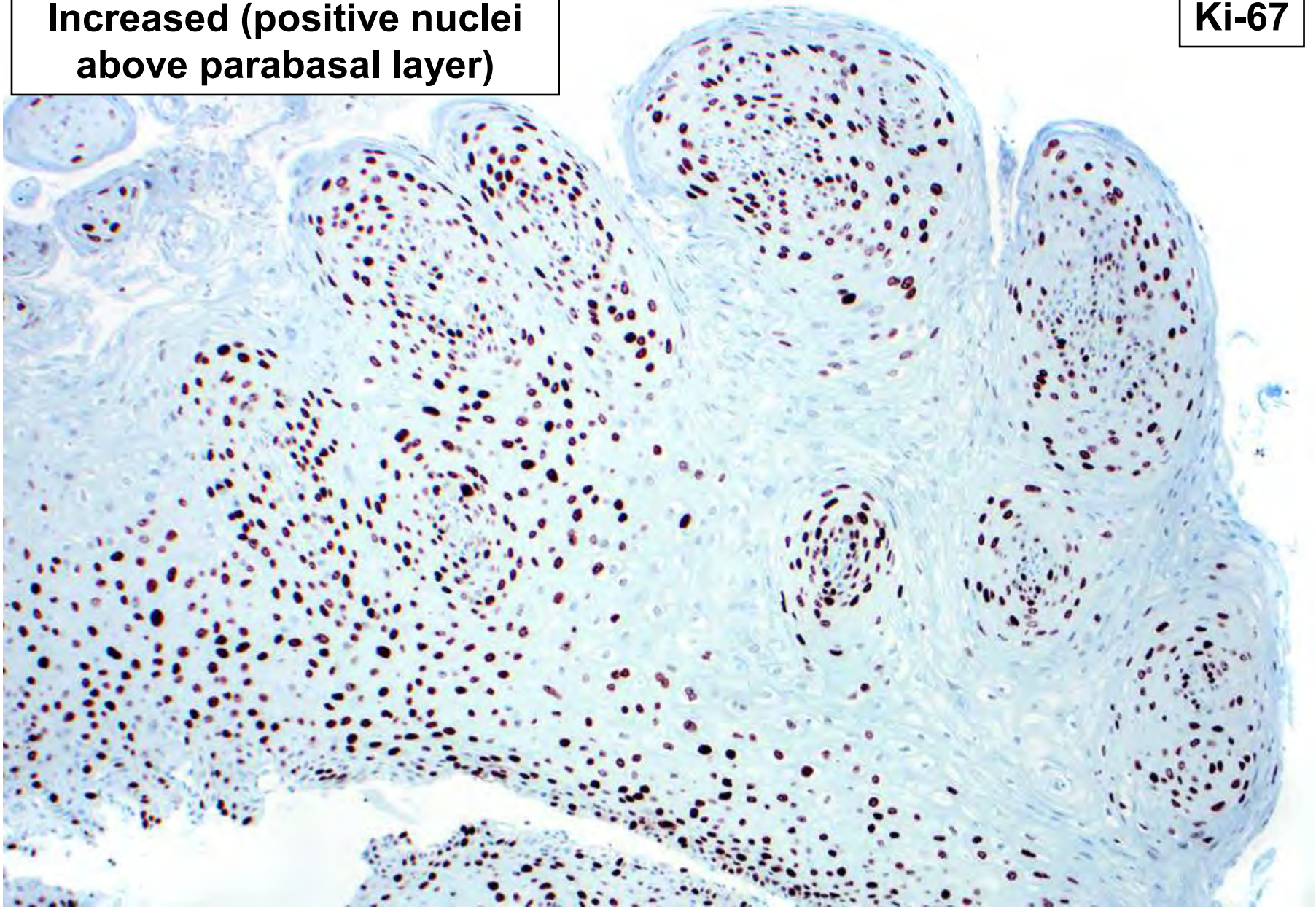
Focal/patchy weak-moderate expression = “Negative”

p16

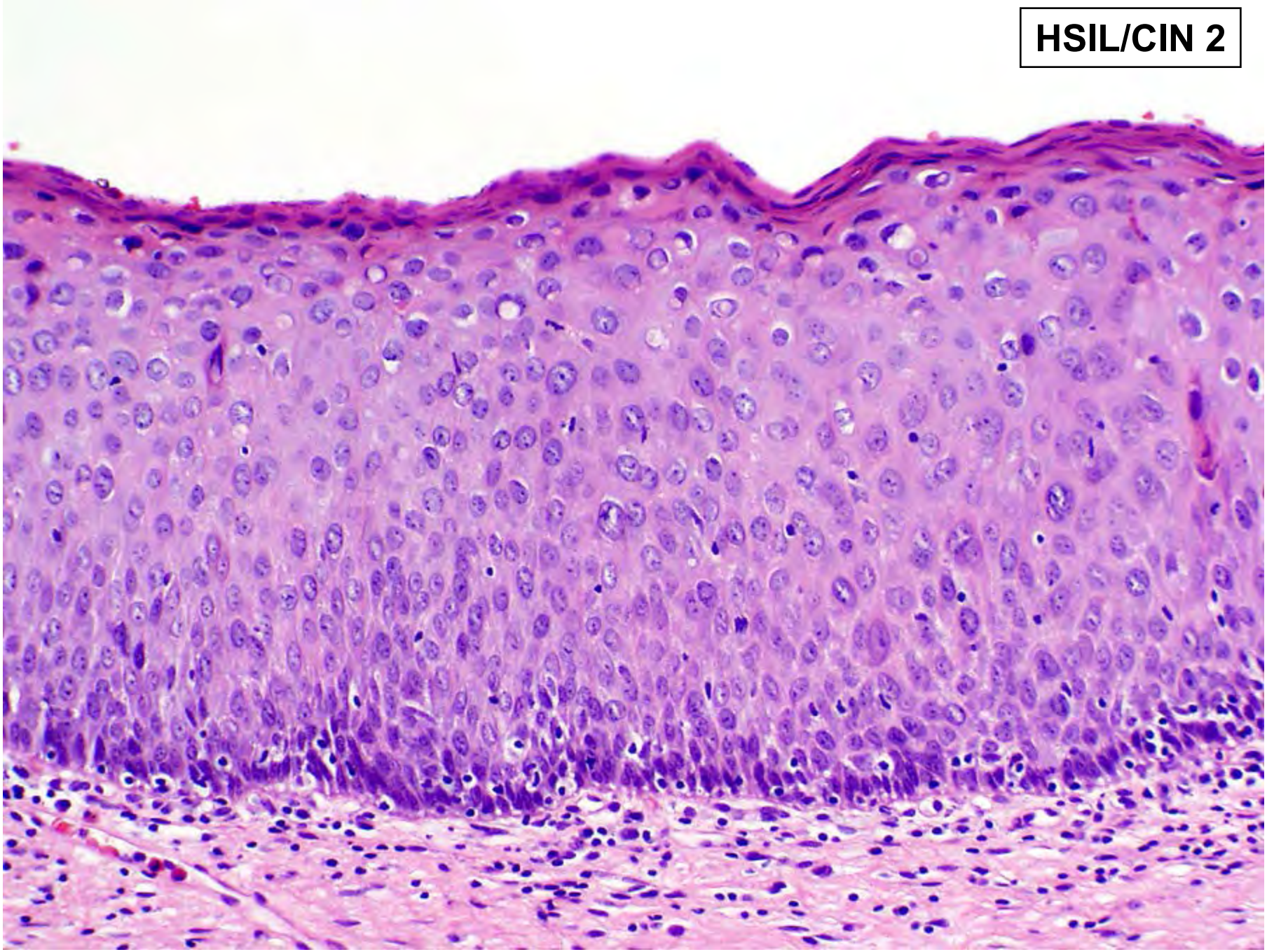


Increased (positive nuclei above parabasal layer)

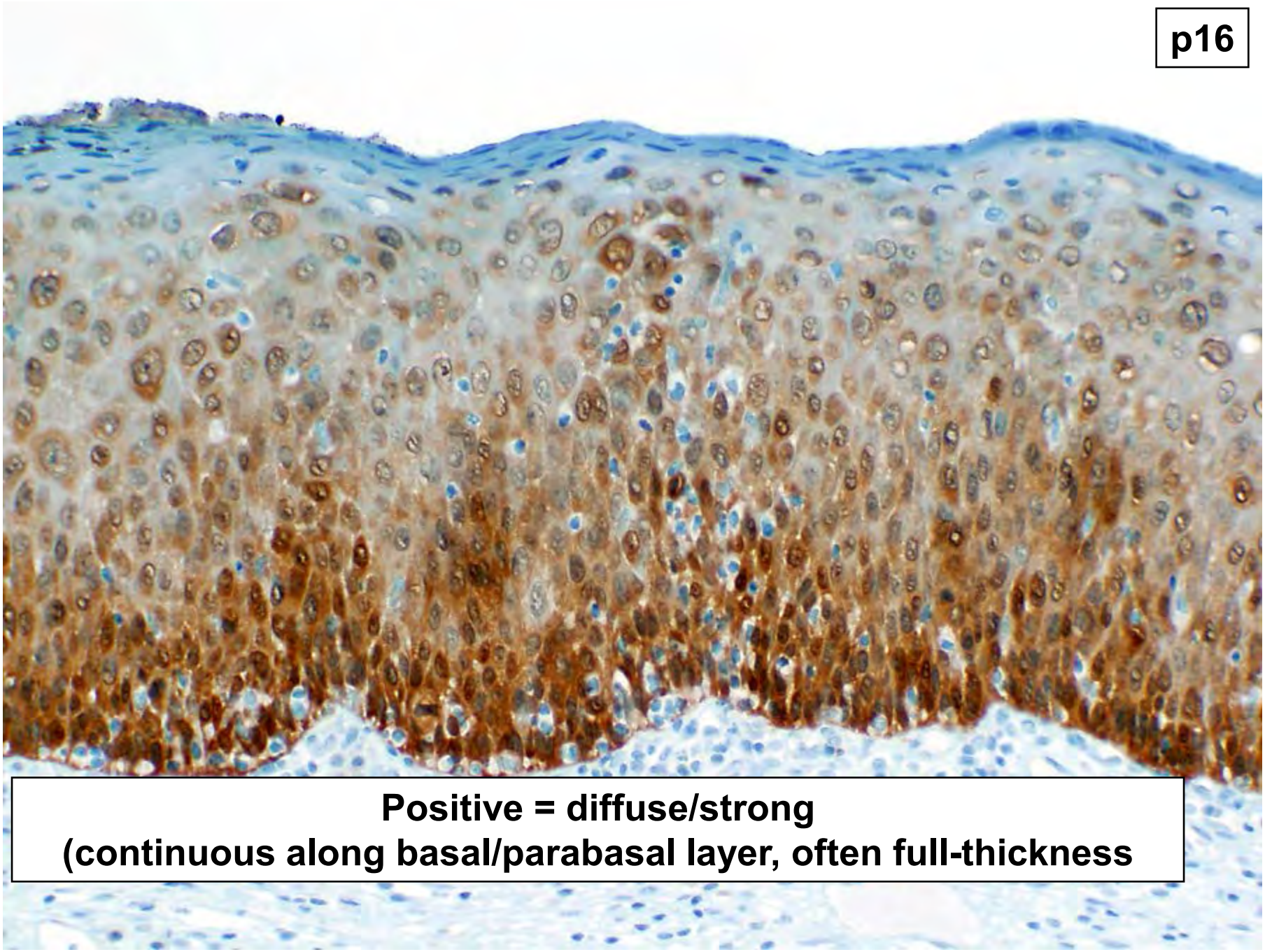
Ki-67



HSIL/CIN 2



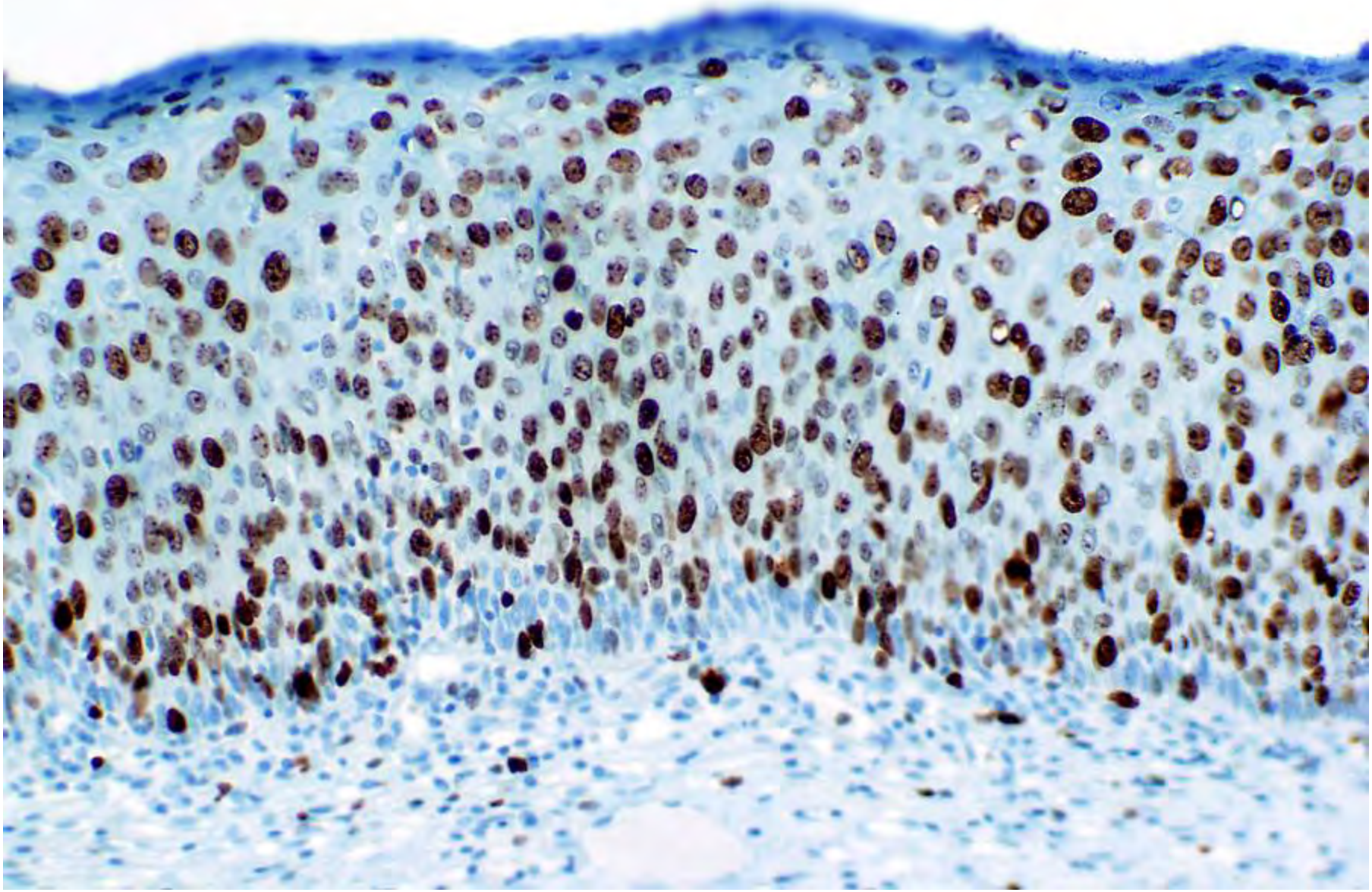
p16

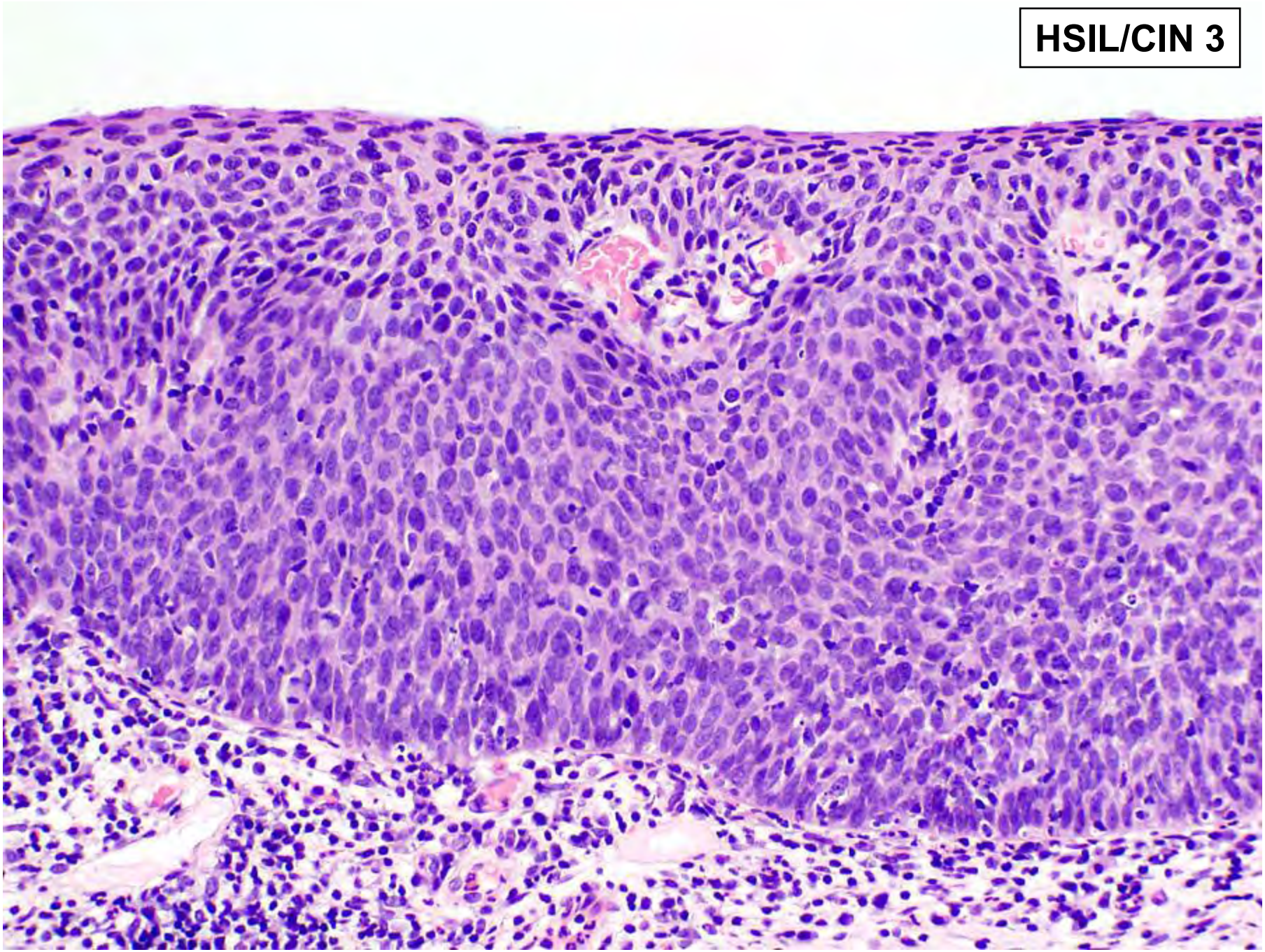


Positive = diffuse/strong
(continuous along basal/parabasal layer, often full-thickness

Increased (positive nuclei in all layers)

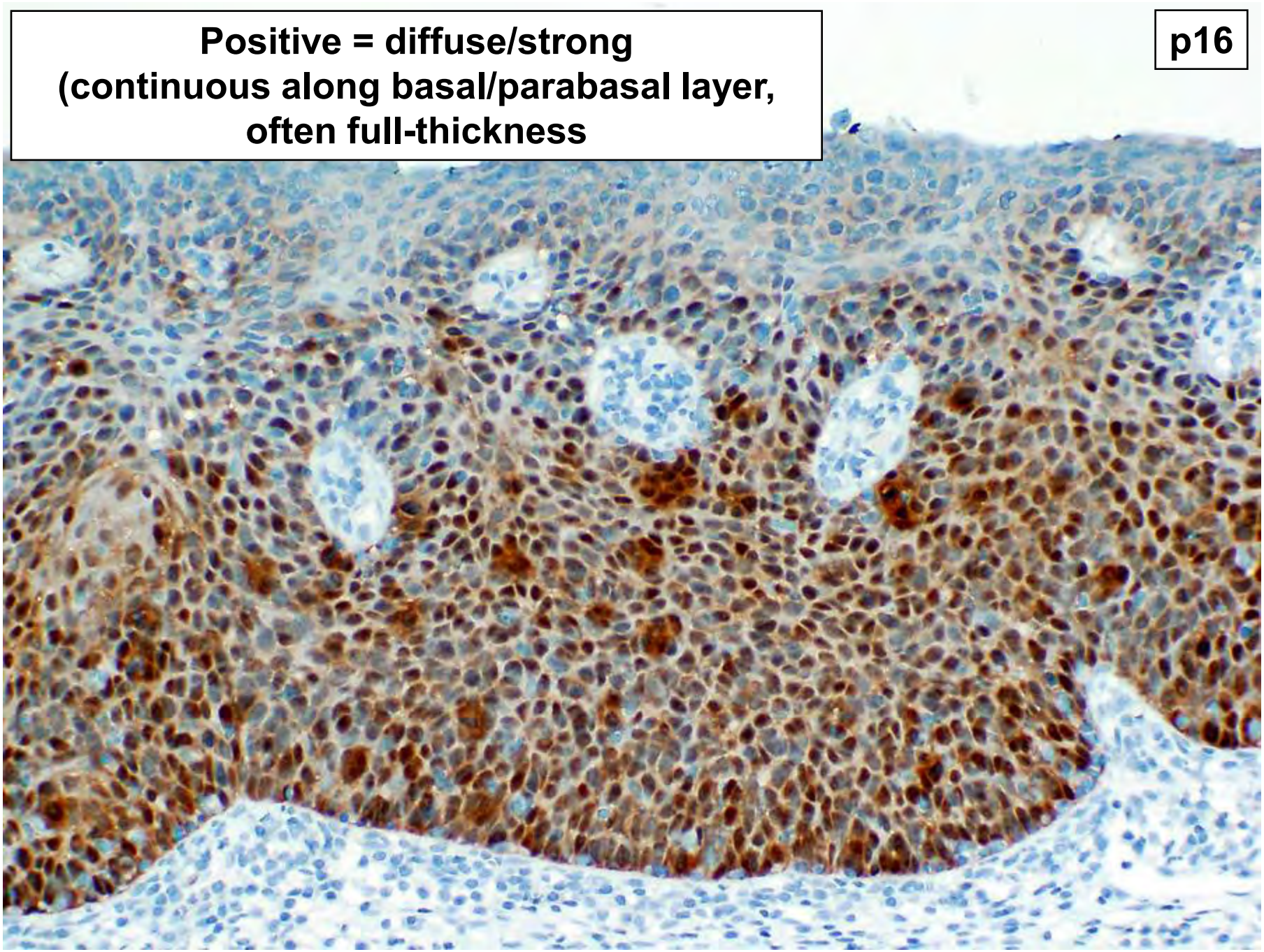
Ki-67




HSIL/CIN 3

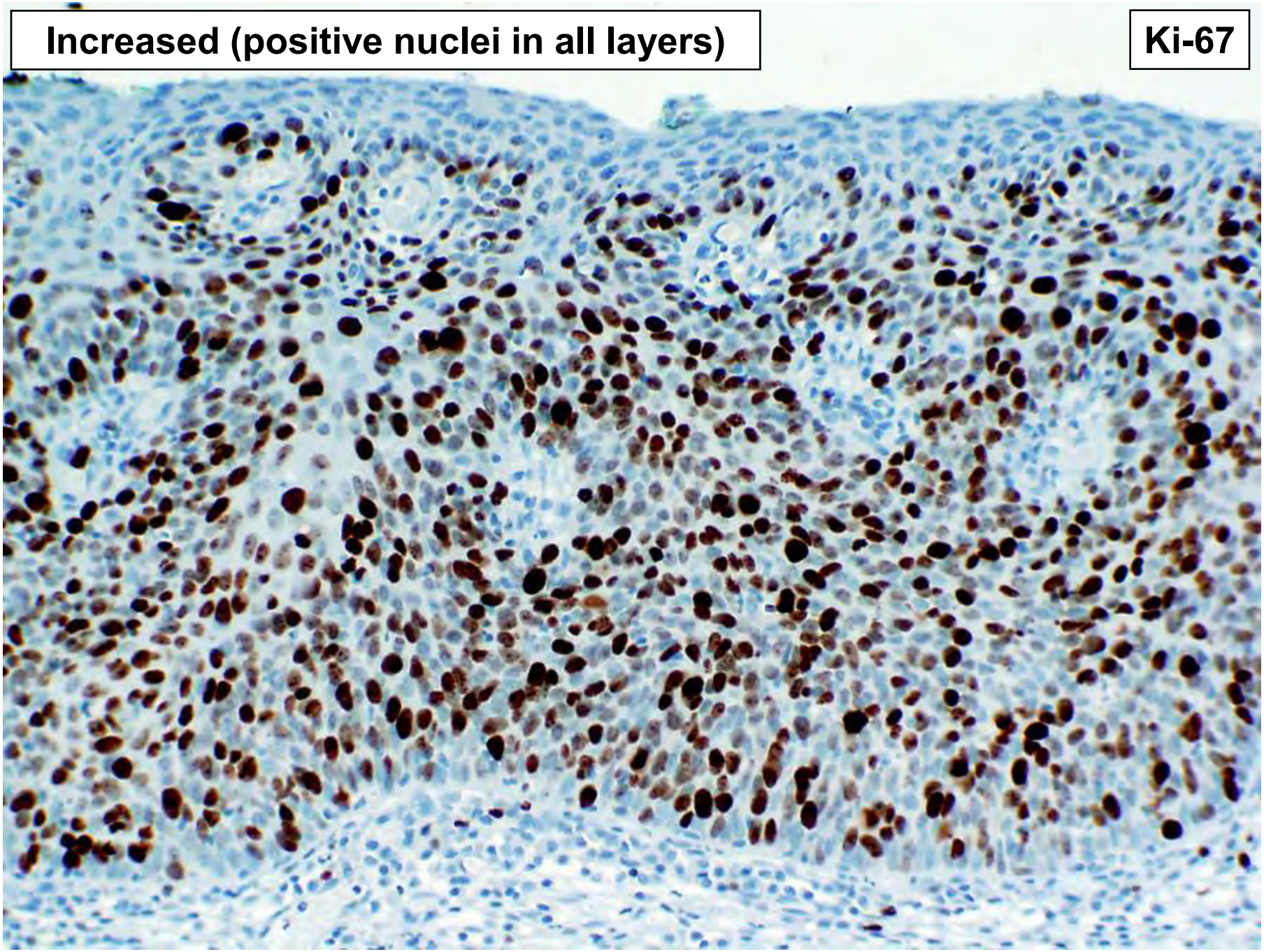
Positive = diffuse/strong
(continuous along basal/parabasal layer, often full-thickness

p16



Increased (positive nuclei in all layers)

Ki-67



Squamous Intraepithelial Lesions: Biomarker Patterns

Diagnosis	p16 –/focal+	p16 diffuse+	Ki-67
NIL	**~95%**	**~5%**	**-- (few ↑)**
LSIL/CIN 1	**~50-60%**	**~40-50%**	**↑ (variable)**
HSIL/CIN 2	**~20-25%**	**~75-80%**	**↑ (few low)**
HSIL/CIN 3	**~1%**	**~99%**	**↑↑ (rare low)**
Atypical immature metaplasia*	*	*	*

* Depends on whether reactive or HSIL (use stains to resolve ddx)

Utility of p16 in Diagnosis of Cervical Squamous Intraepithelial Lesions

- **For distinction of precancer (HSIL/CIN 2&3) from mimickers of precancer:**
  - **HSIL: p16 diffuse+ (“block” staining)**
  - **Mimickers of HSIL: p16 negative/patchy**
    - **Reactive/inflammatory changes**
    - **Atypical immature metaplasia (“AIM”)**
    - **Atrophy**
    - **Squamo-transitional metaplasia**

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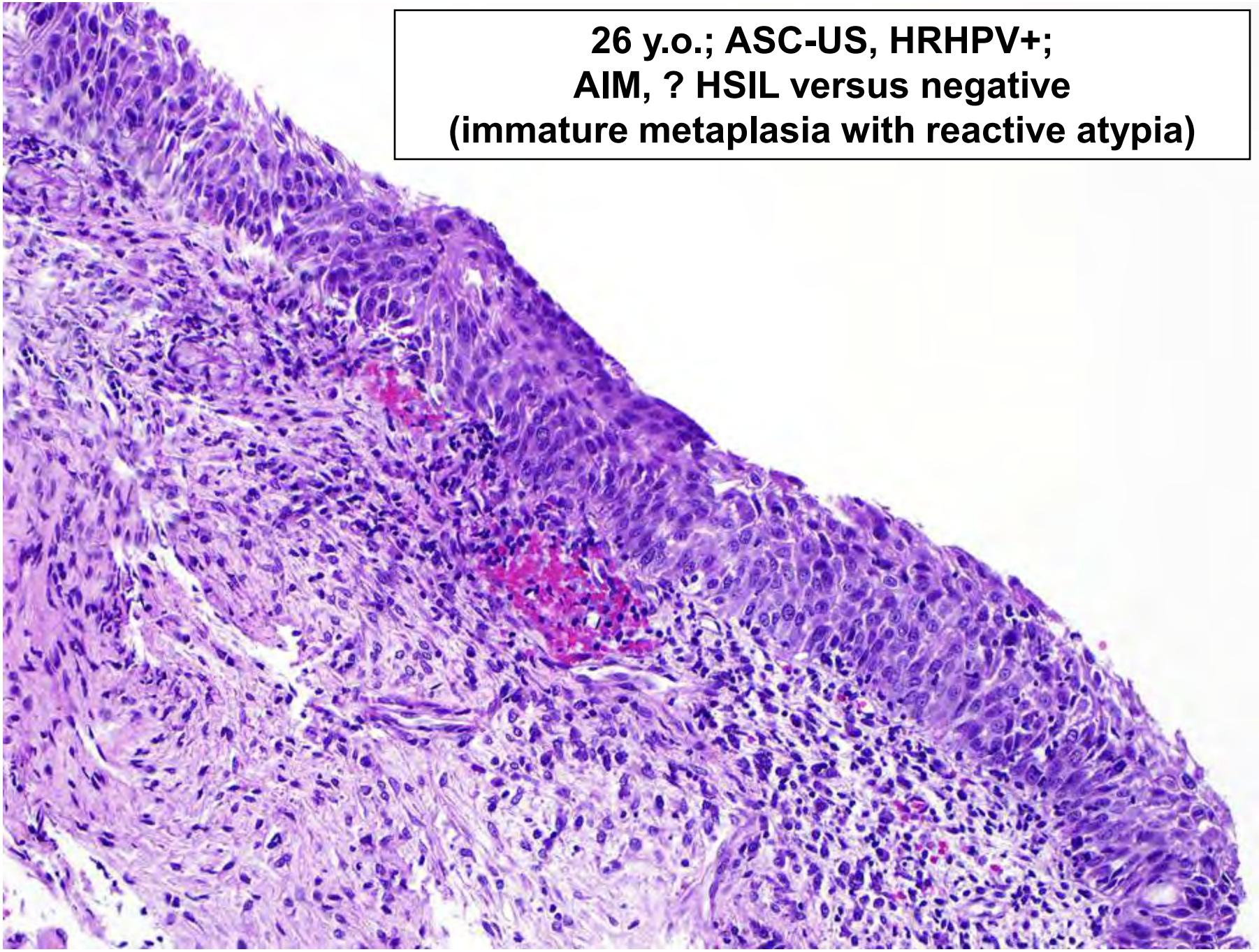
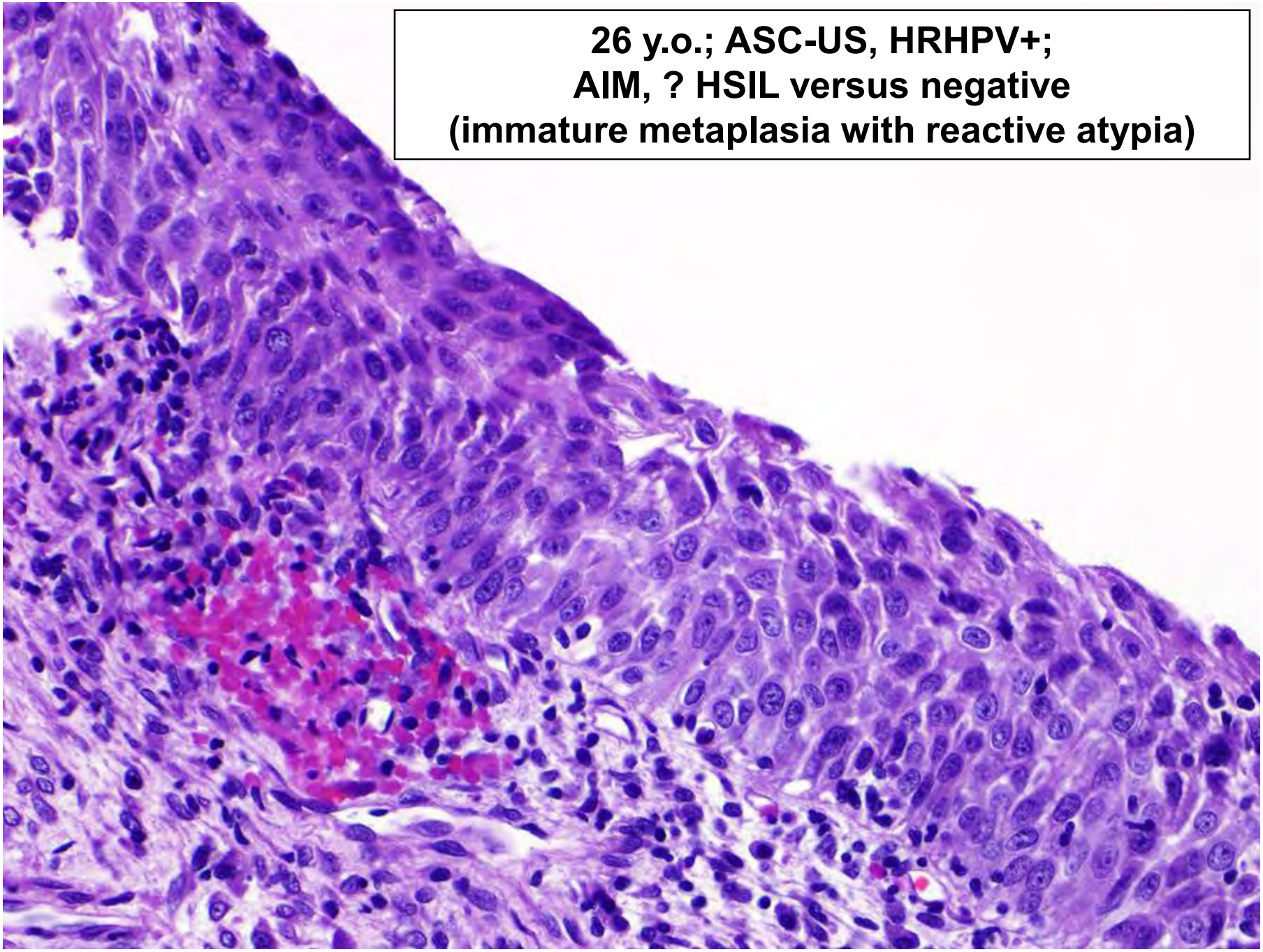

26 y.o.; ASC-US, HRHPV+;
AIM, ? HSIL versus negative
(immature metaplasia with reactive atypia)


26 y.o.; ASC-US, HRHPV+;
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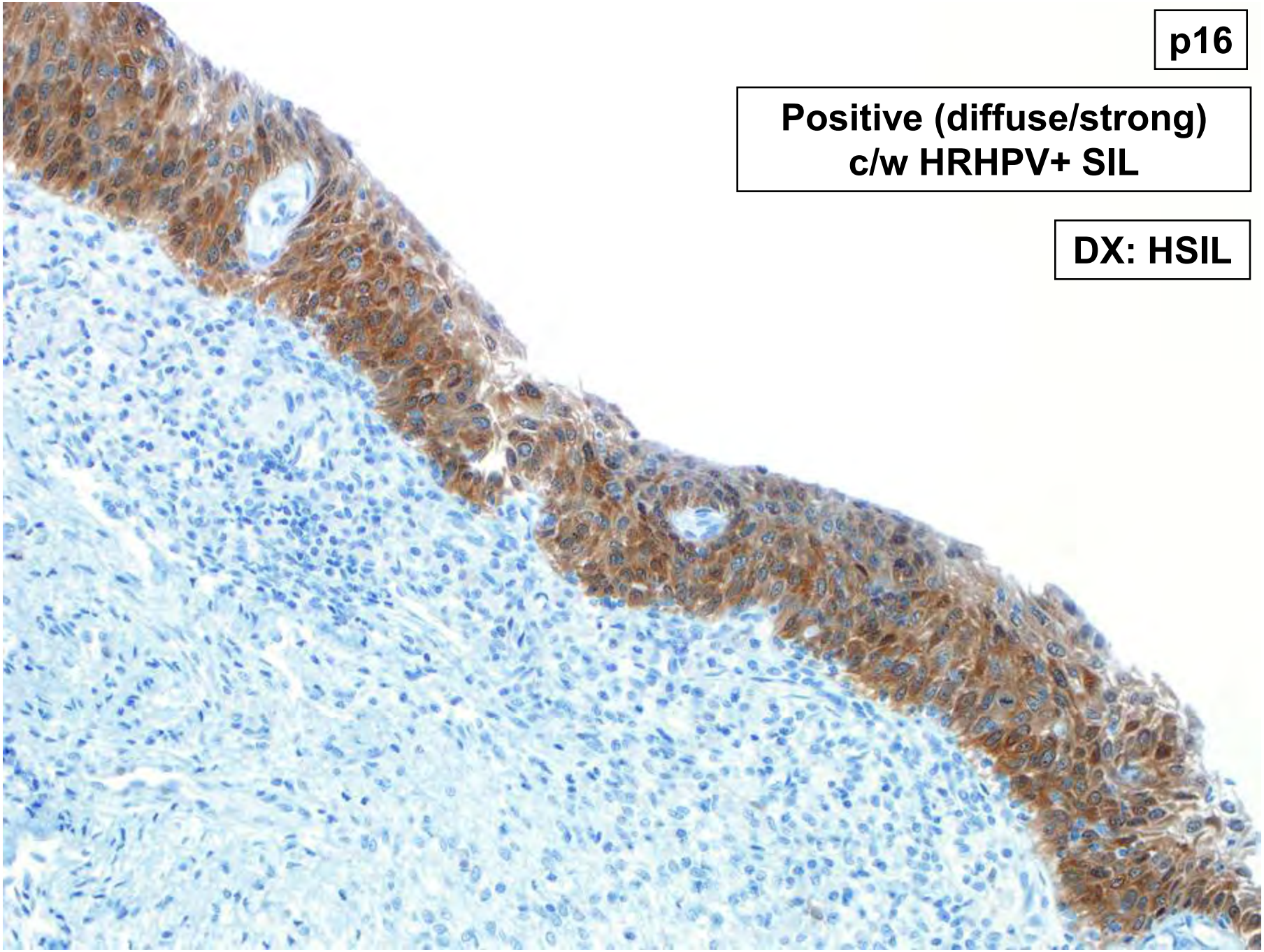
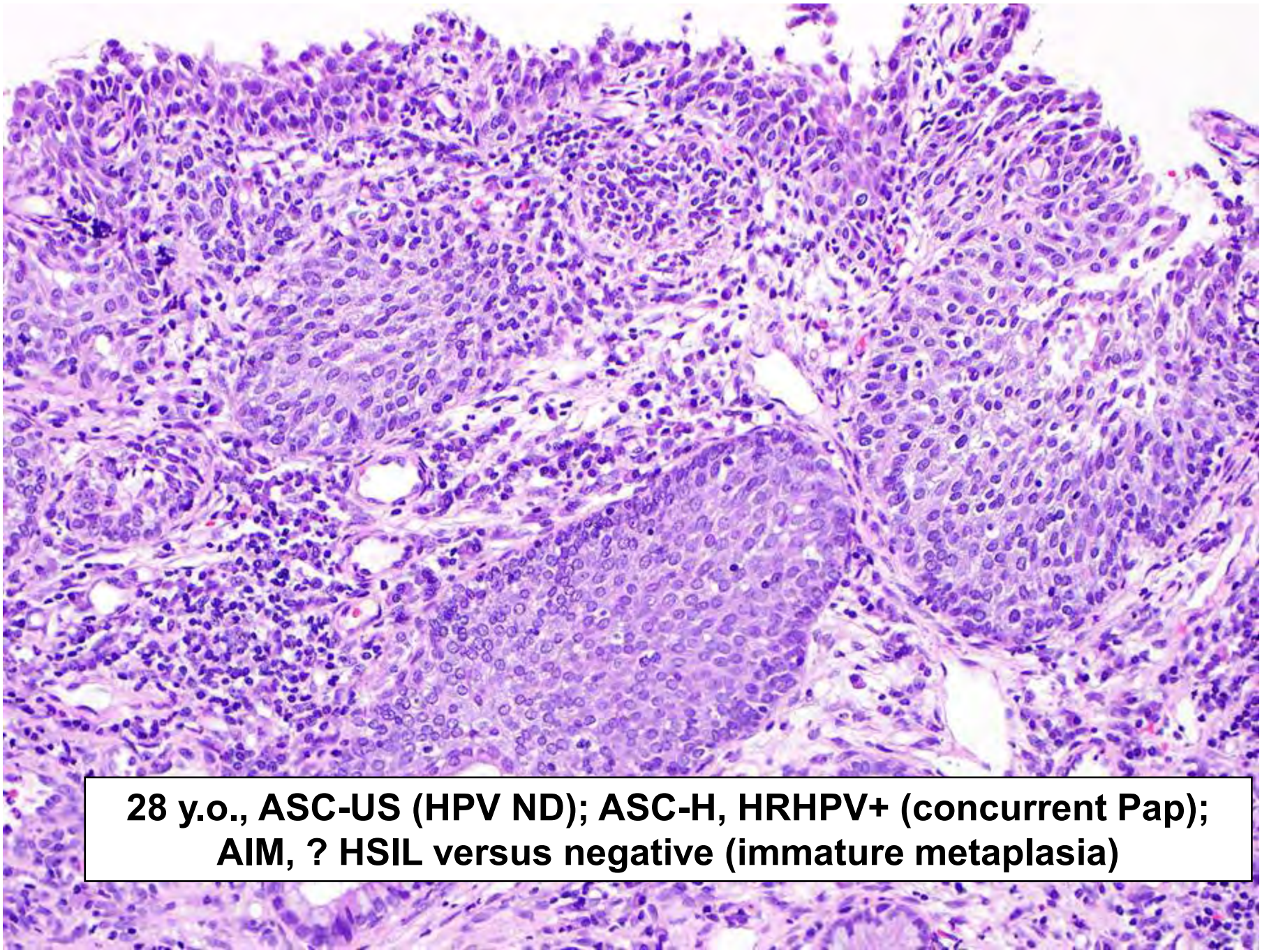
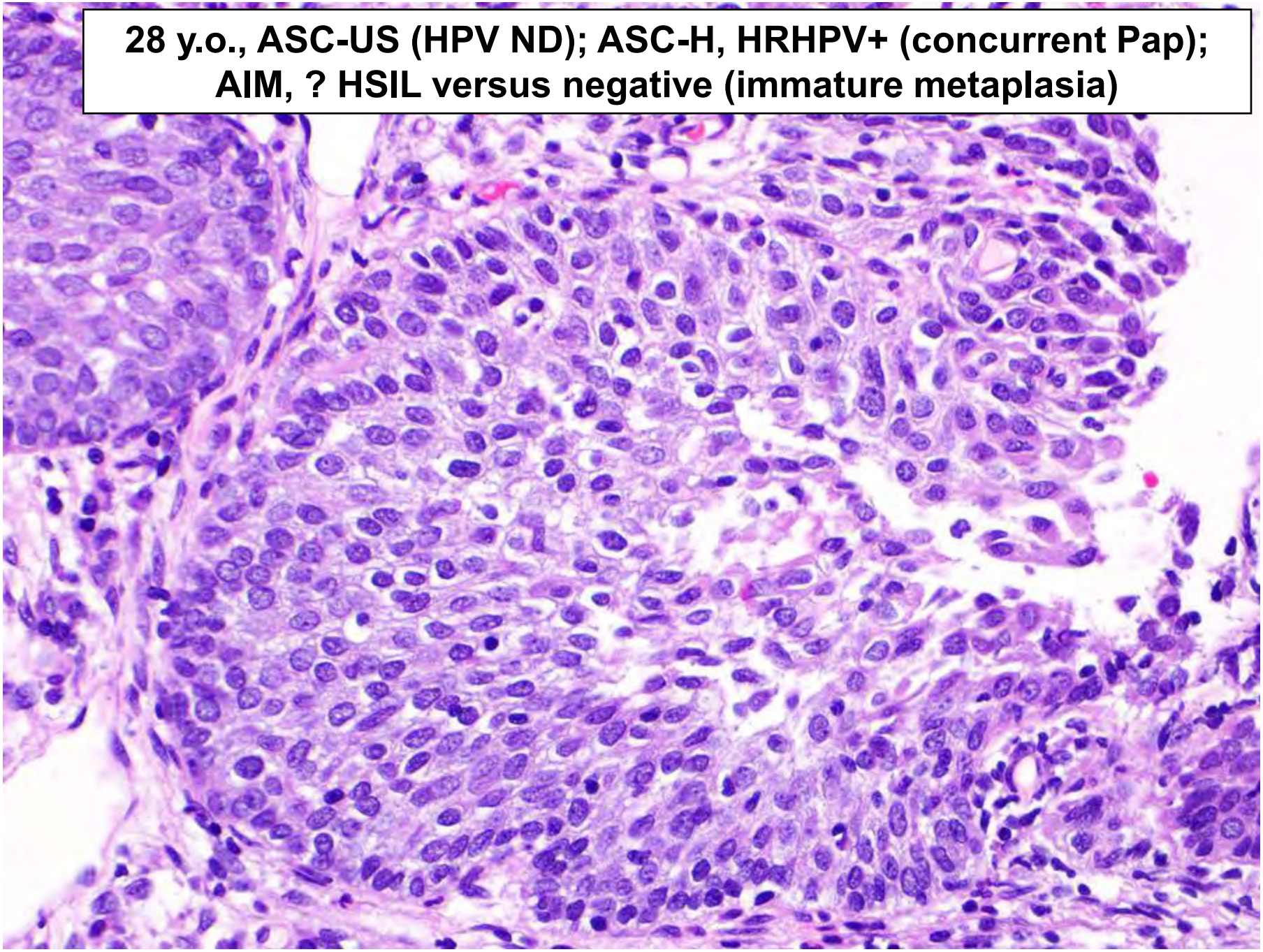
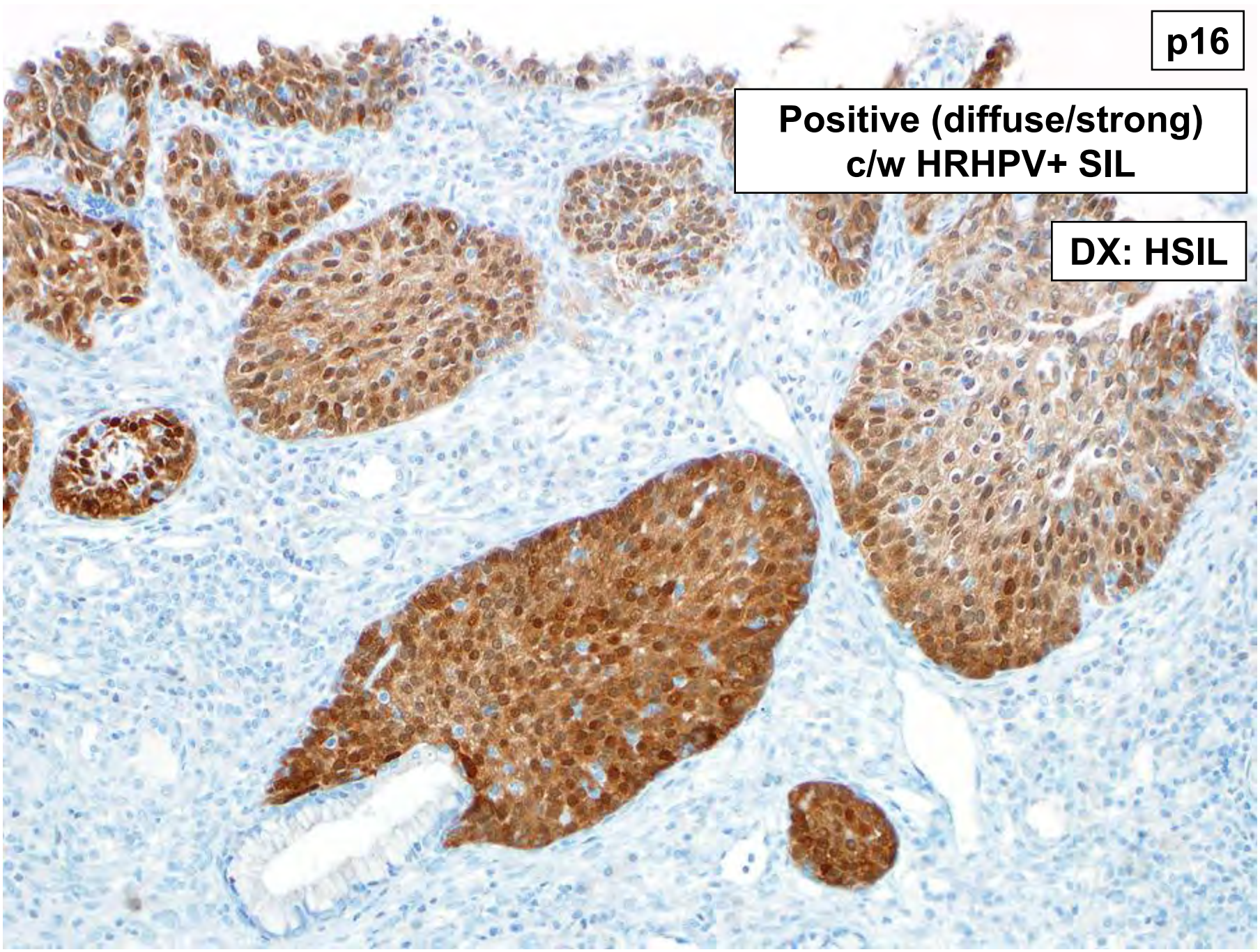
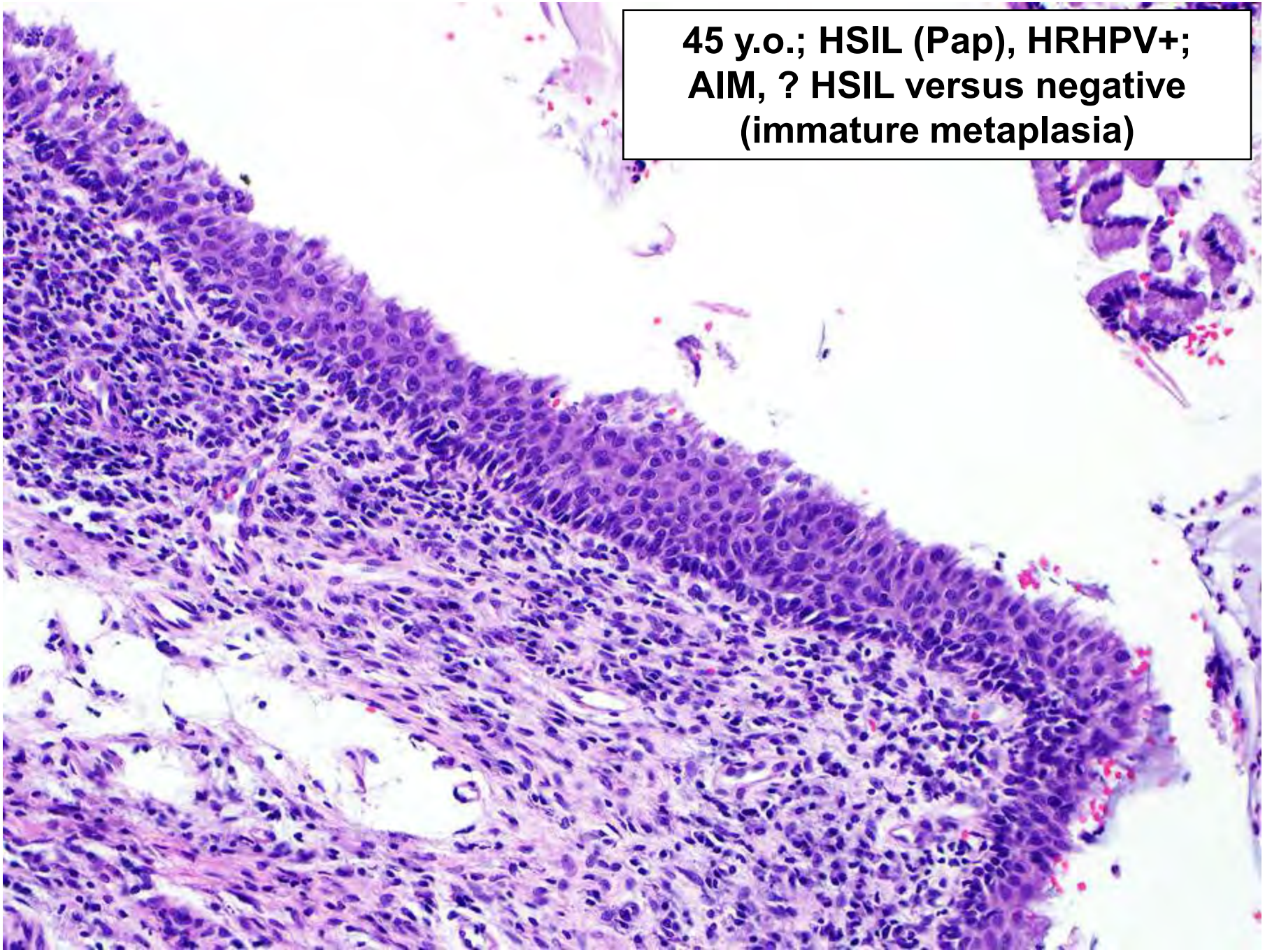
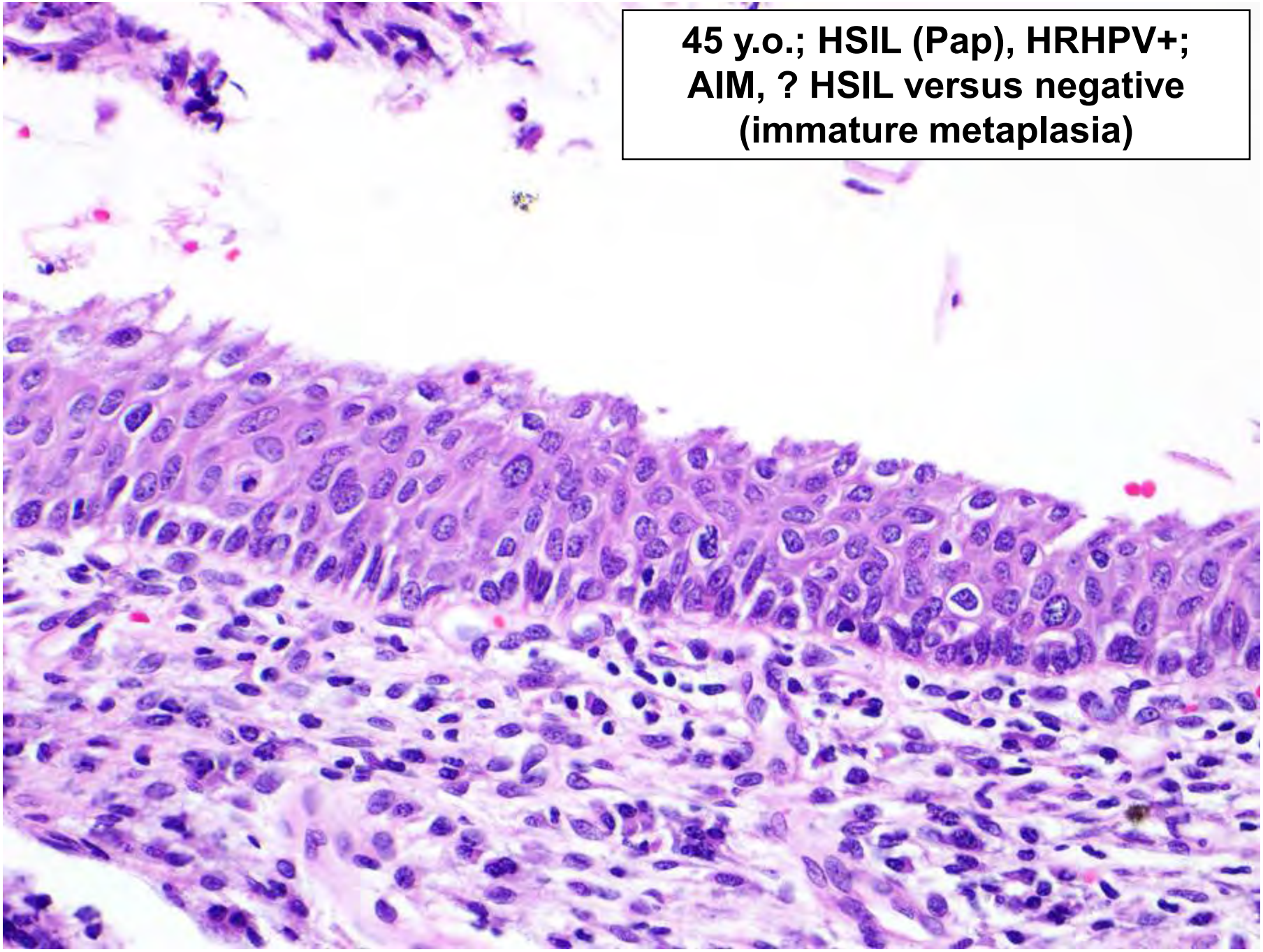

p16
Positive (diffuse/strong)
c/w HRHPV+ SIL
DX: HSIL

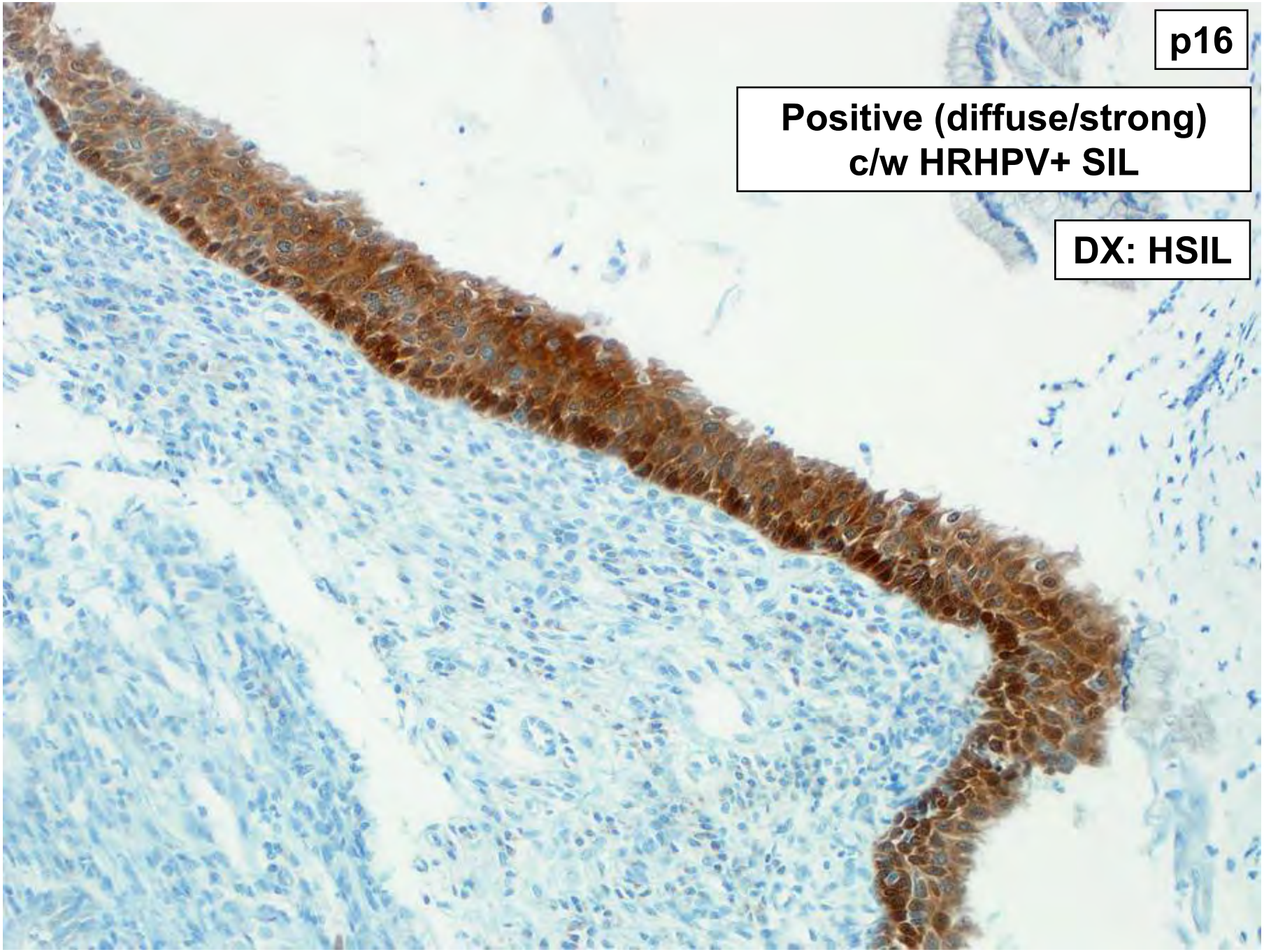

28 y.o., ASC-US (HPV ND); ASC-H, HRHPV+ (concurrent Pap);
AIM, ? HSIL versus negative (immature metaplasia)


28 y.o., ASC-US (HPV ND); ASC-H, HRHPV+ (concurrent Pap);
AIM, ? HSIL versus negative (immature metaplasia)

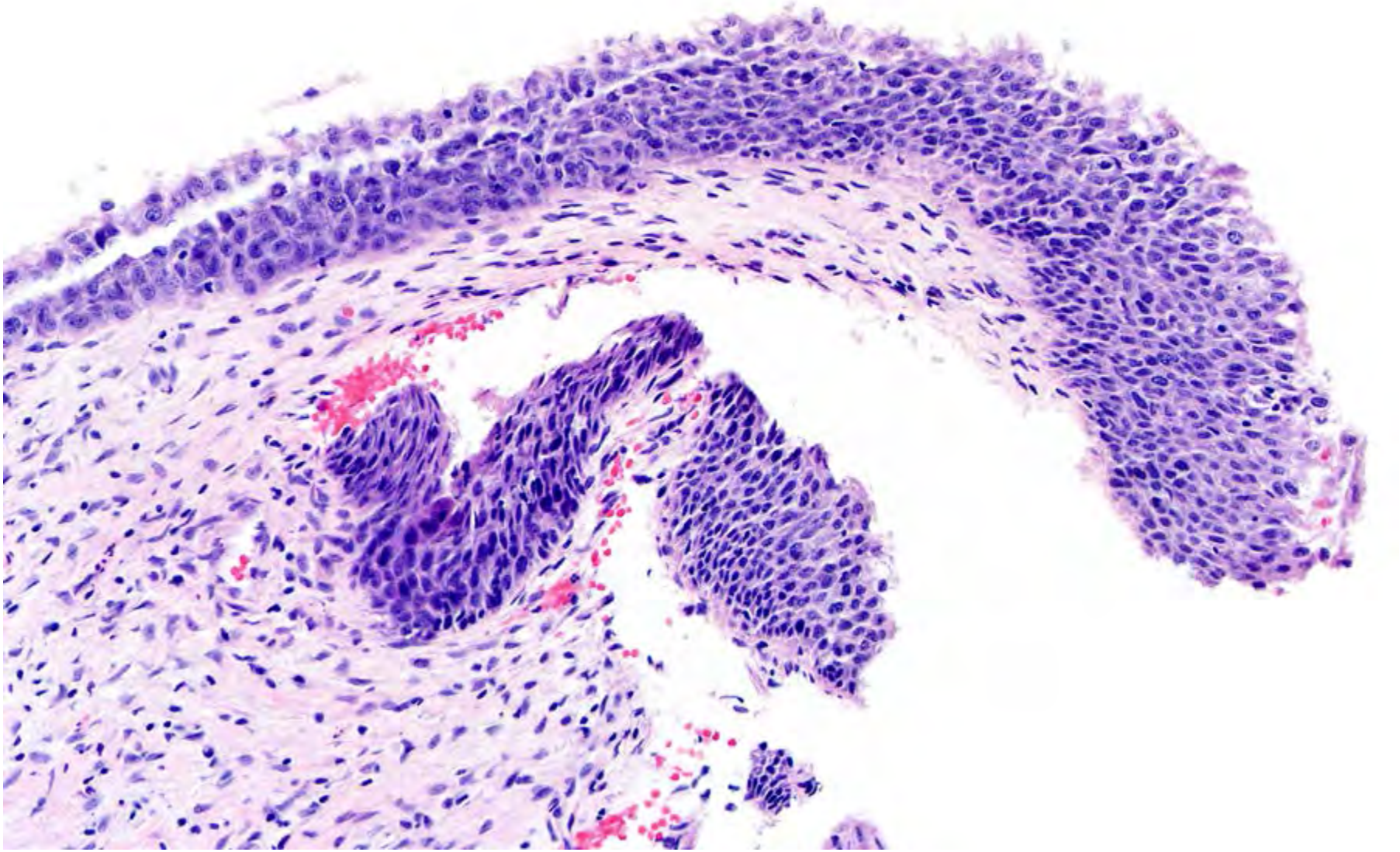

p16
Positive (diffuse/strong)
c/w HRHPV+ SIL
DX: HSIL

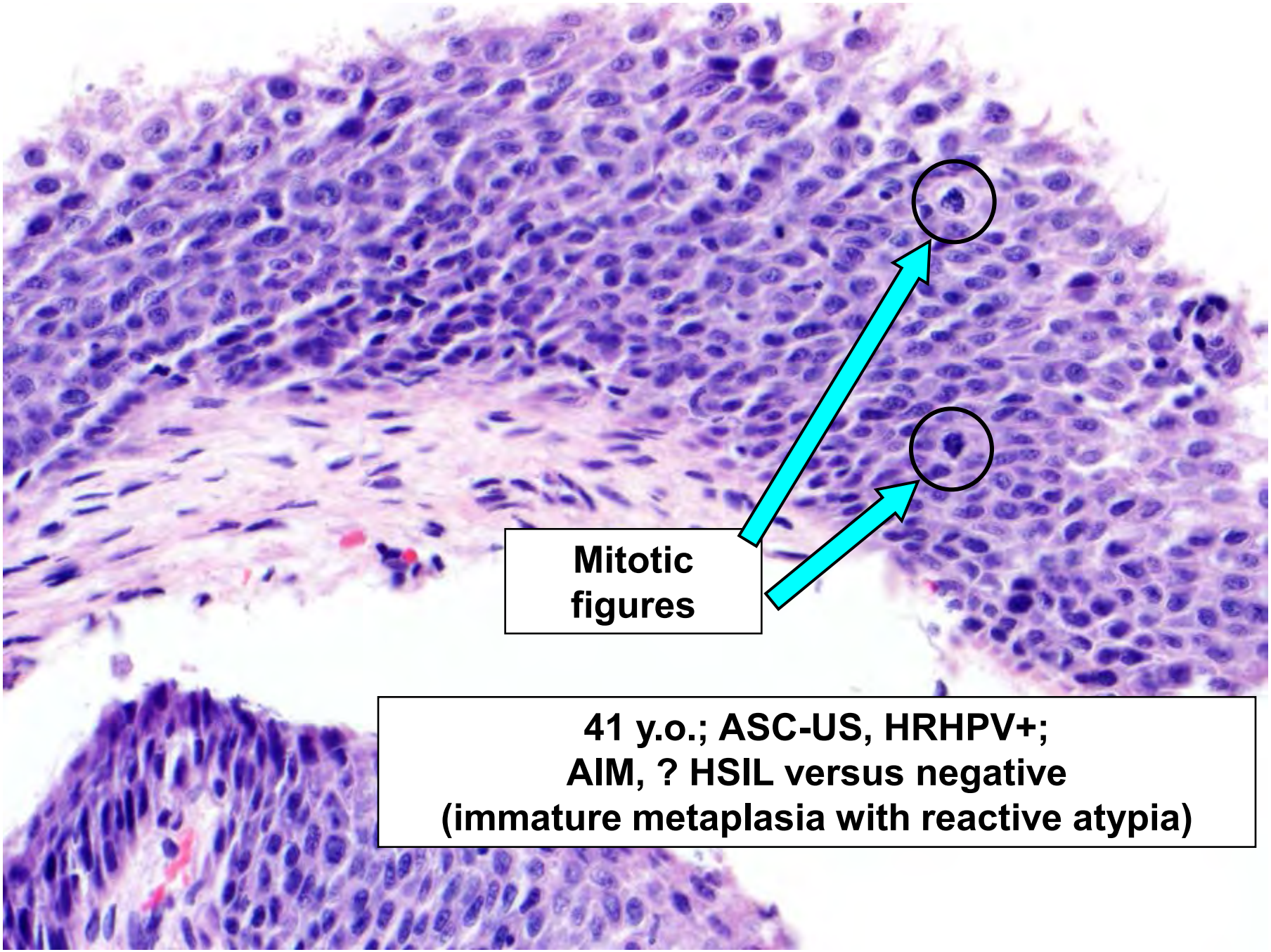

45 y.o.; HSIL (Pap), HRHPV+;
AIM, ? HSIL versus negative
(immature metaplasia)


45 y.o.; HSIL (Pap), HRHPV+;
AIM, ? HSIL versus negative
(immature metaplasia)

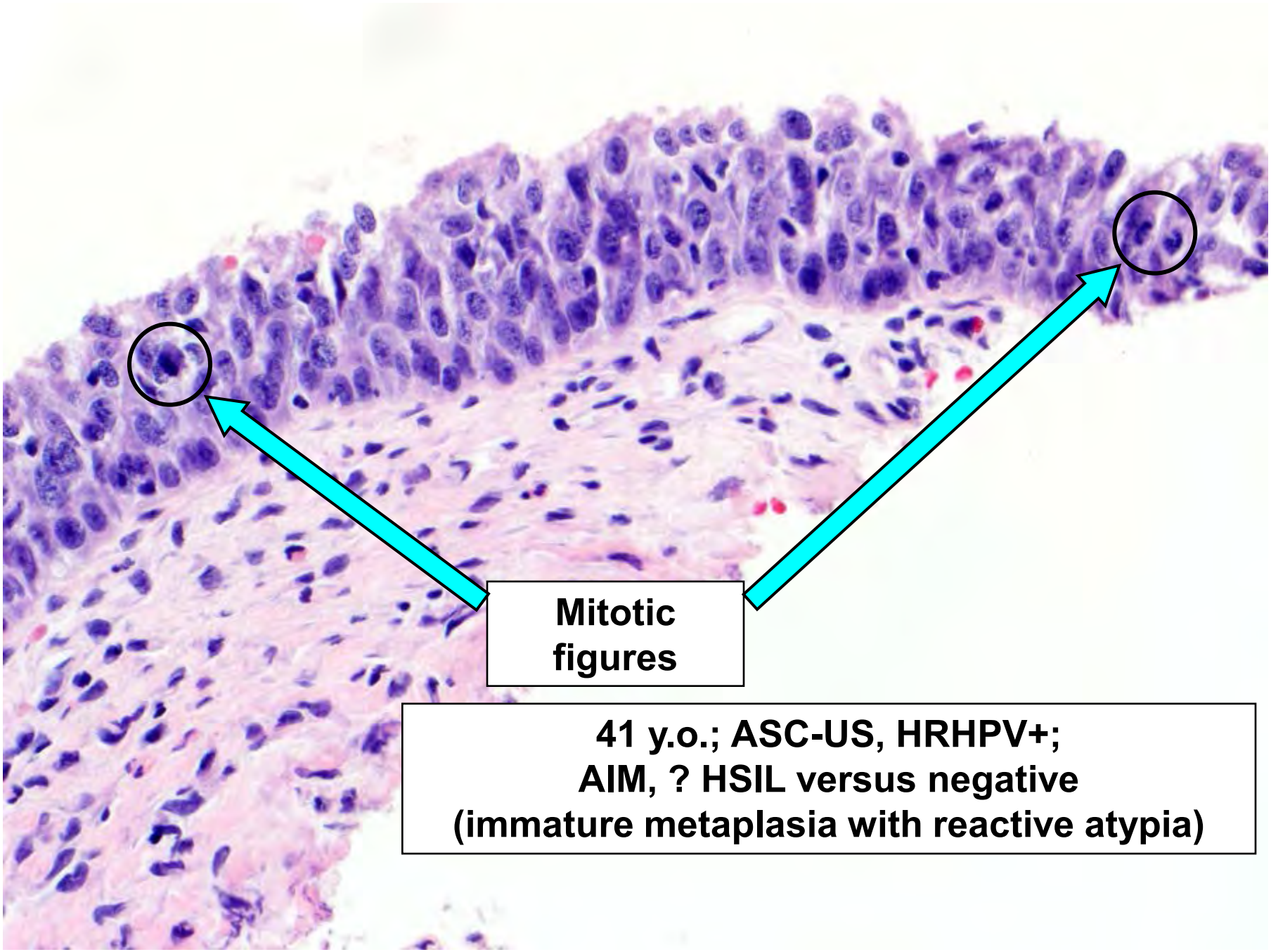

p16
Positive (diffuse/strong)
c/w HRHPV+ SIL
DX: HSIL

41 y.o.; ASC-US, HRHPV+;
AIM, ? HSIL versus negative
(immature metaplasia with reactive atypia)



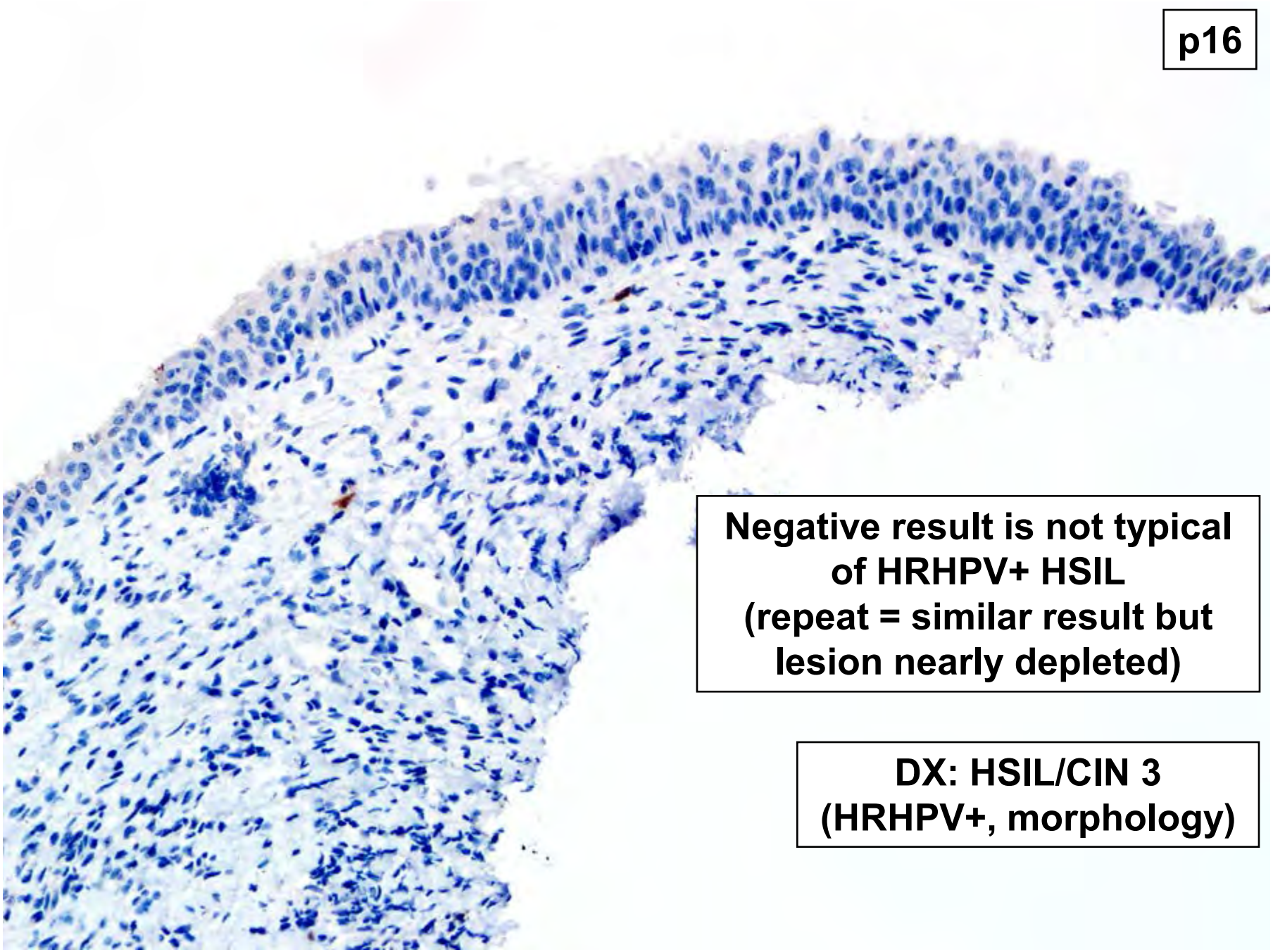


41 y.o.; ASC-US, HRHPV+;
AIM, ? HSIL versus negative
(immature metaplasia with reactive atypia)



41 y.o.; ASC-US, HRHPV+;
AIM, ? HSIL versus negative
(immature metaplasia with reactive atypia)

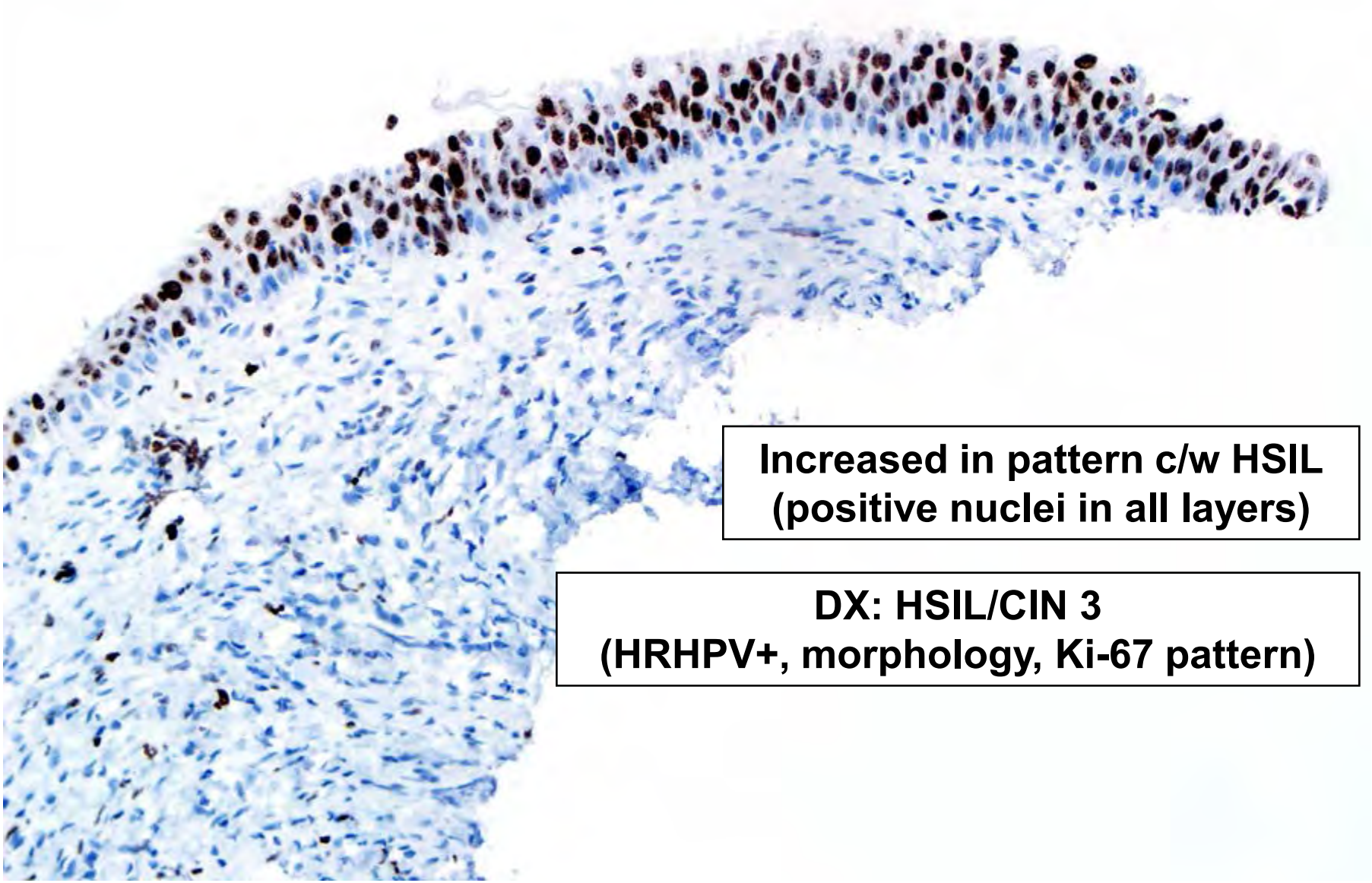
p16



Negative result is not typical of HRHPV+ HSIL (repeat = similar result but lesion nearly depleted)

DX: HSIL/CIN 3 (HRHPV+, morphology)

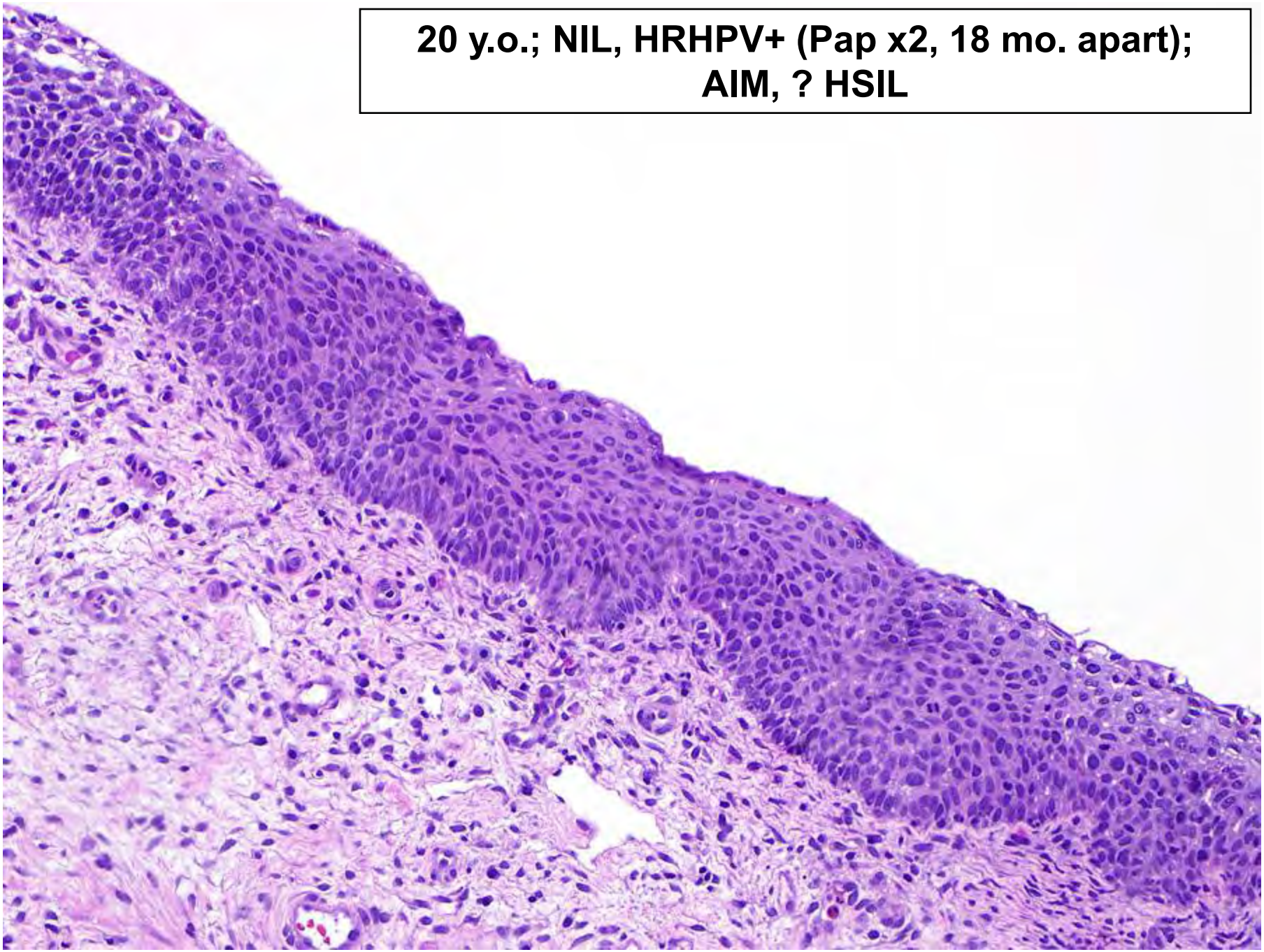
Ki-67

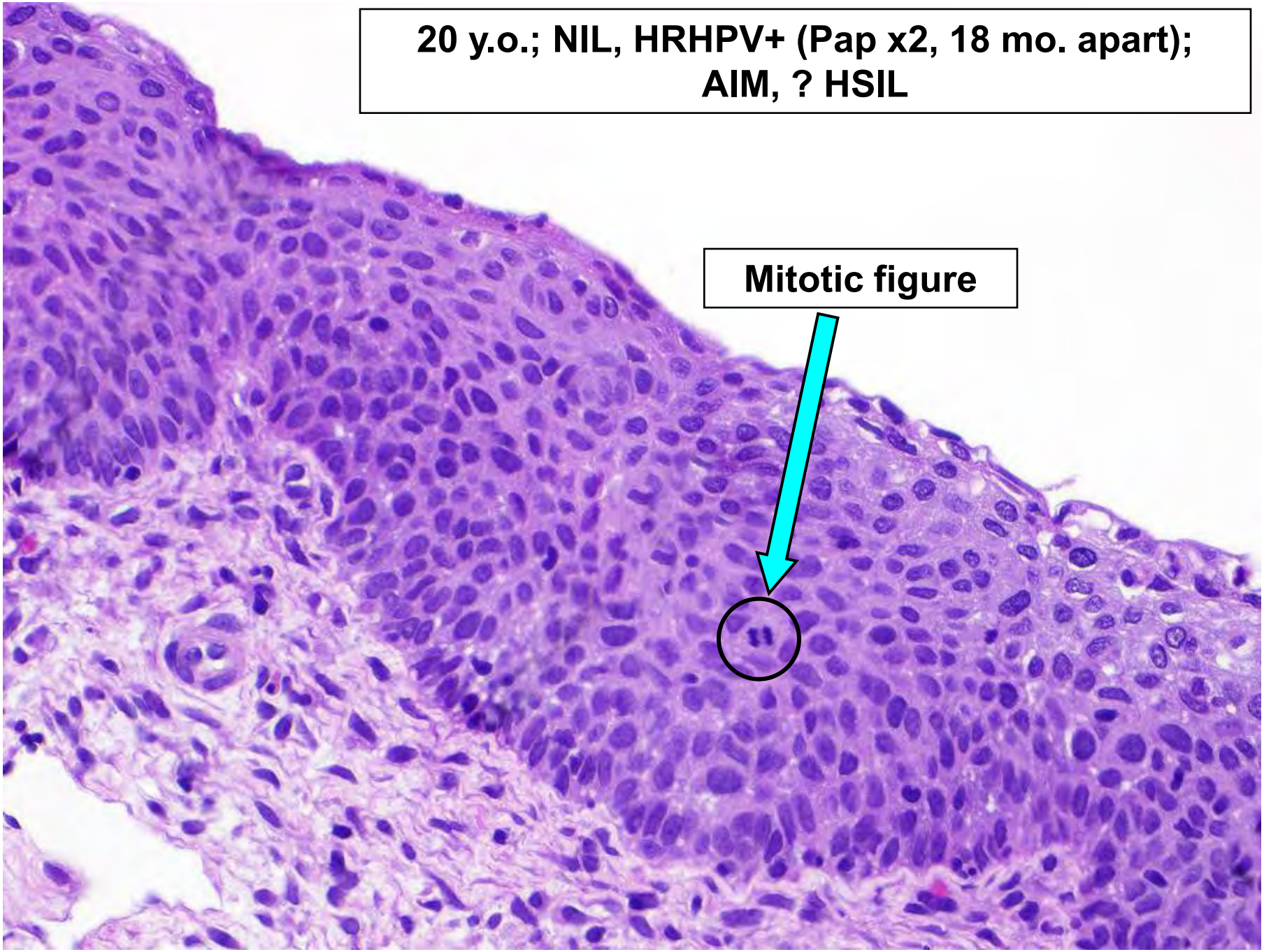


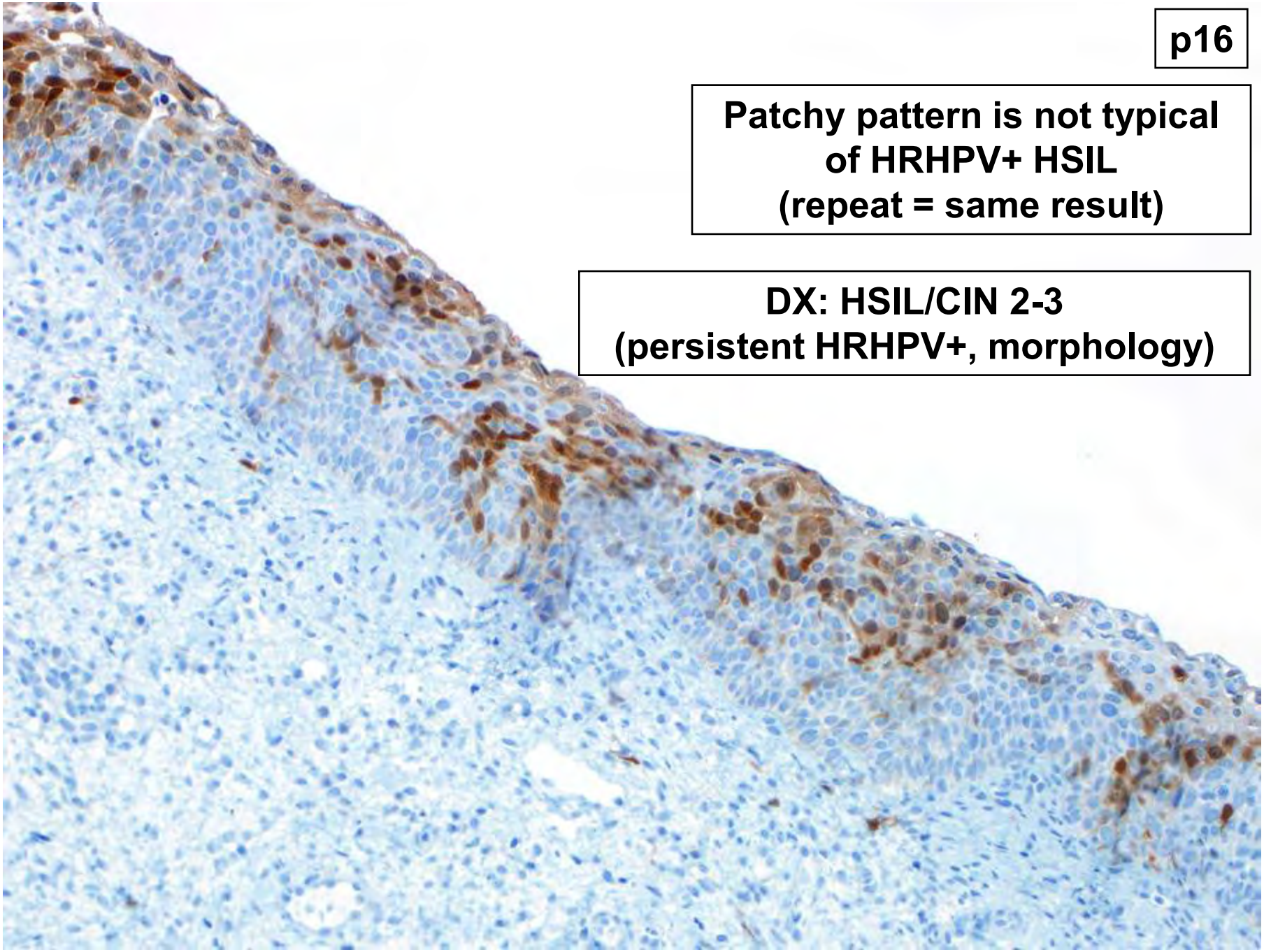
Increased in pattern c/w HSIL
(positive nuclei in all layers)

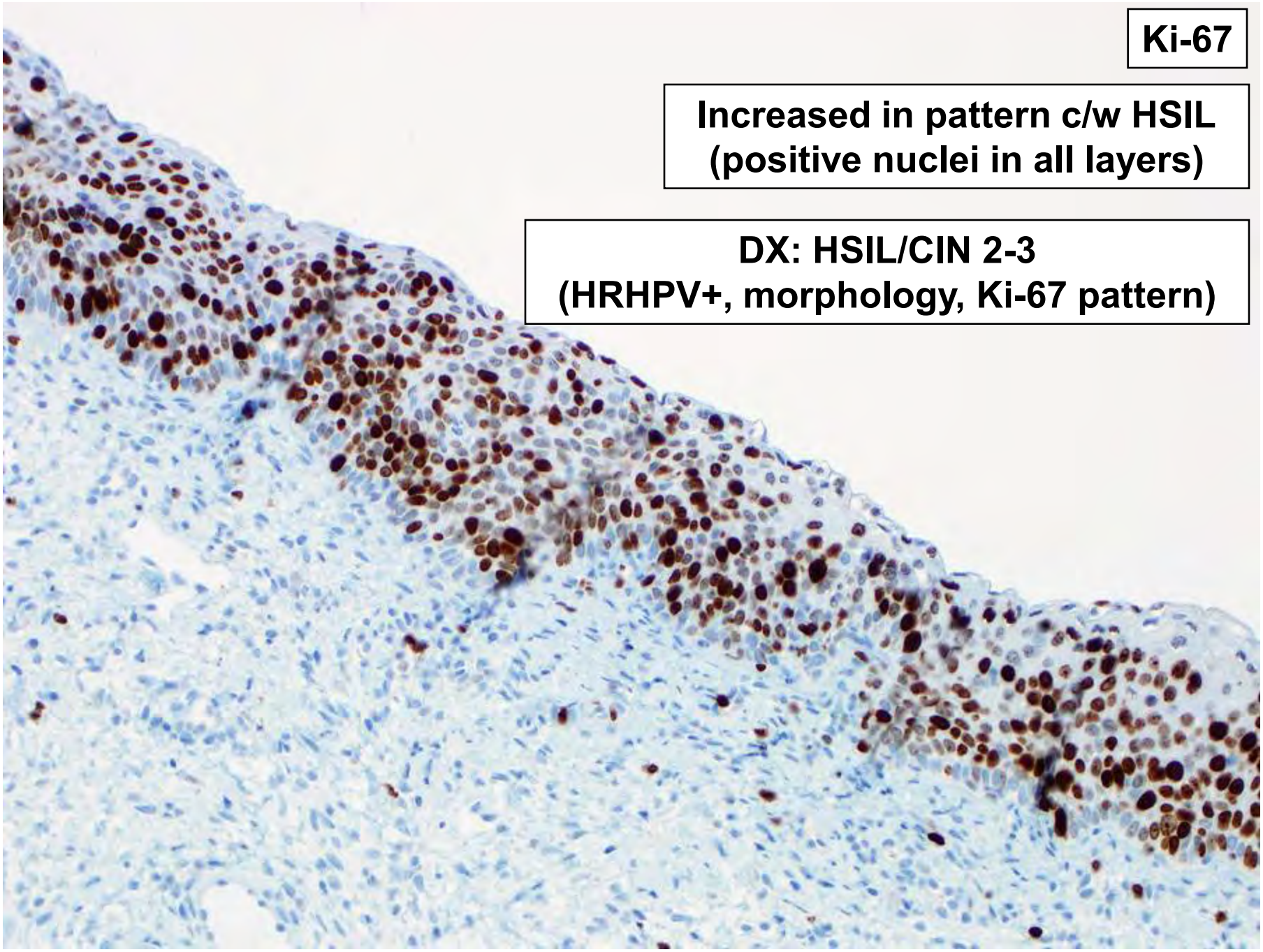
DX: HSIL/CIN 3
(HRHPV+, morphology, Ki-67 pattern)

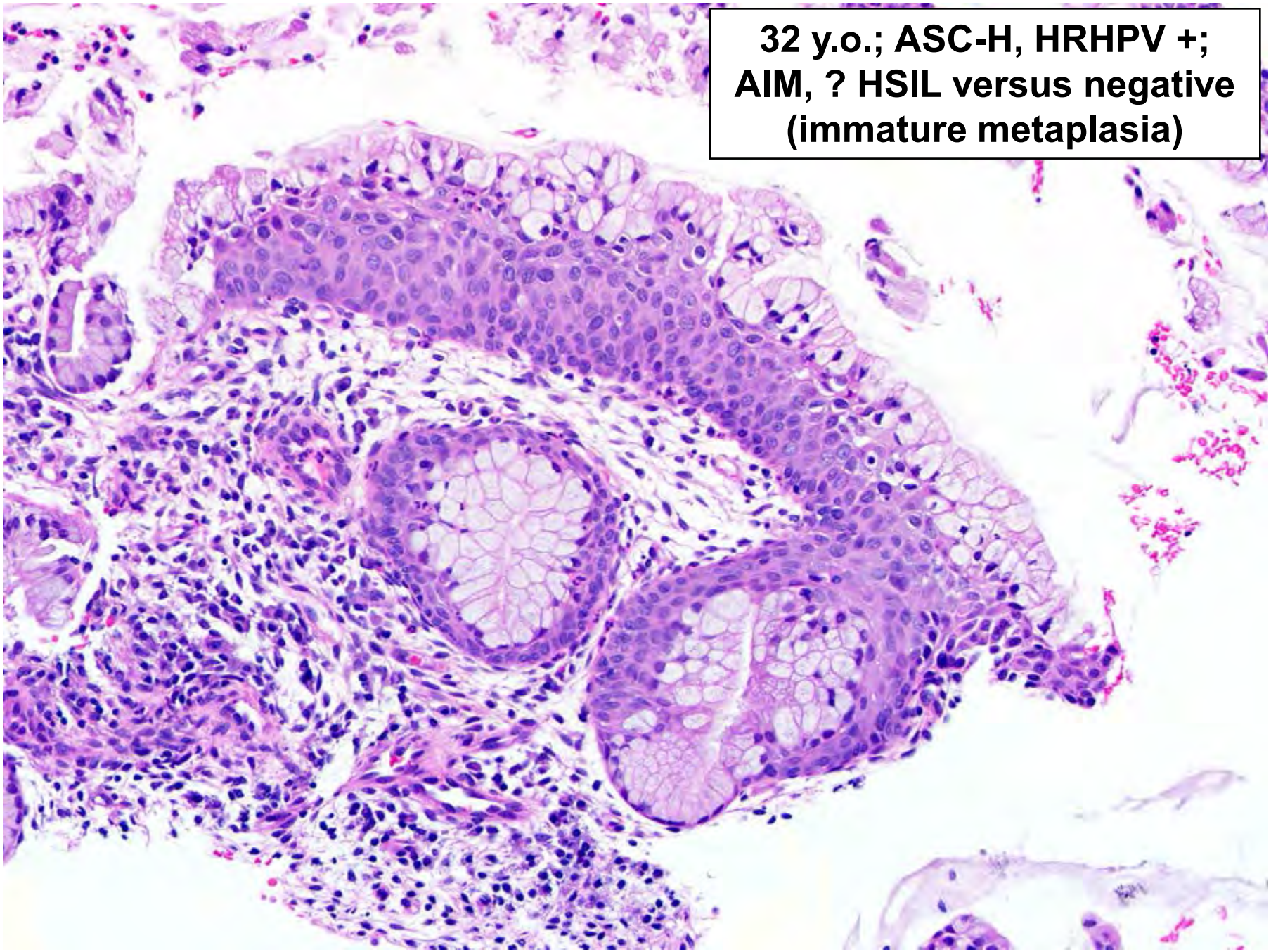
20 y.o.; NIL, HRHPV+ (Pap x2, 18 mo. apart); AIM, ? HSIL

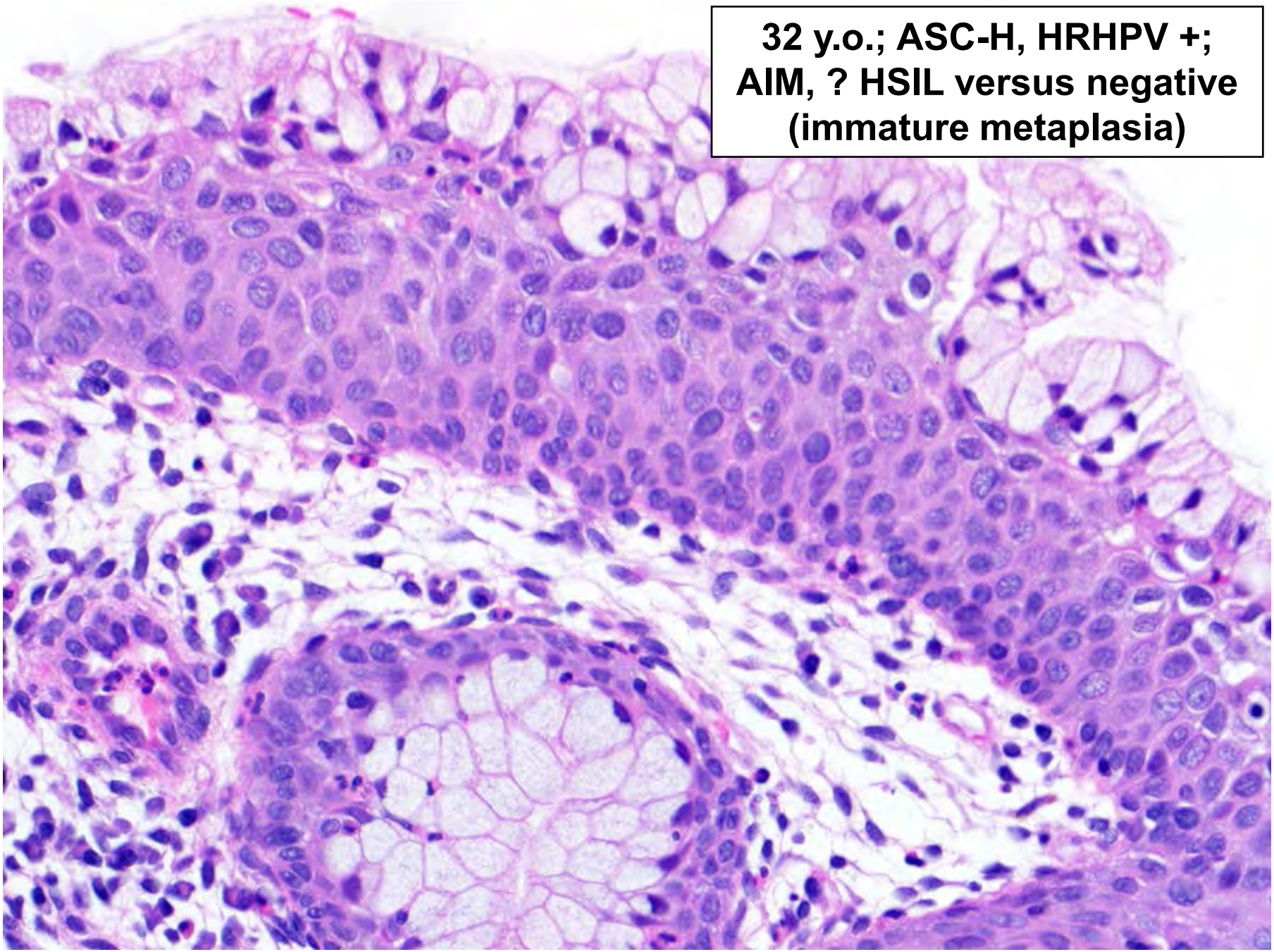


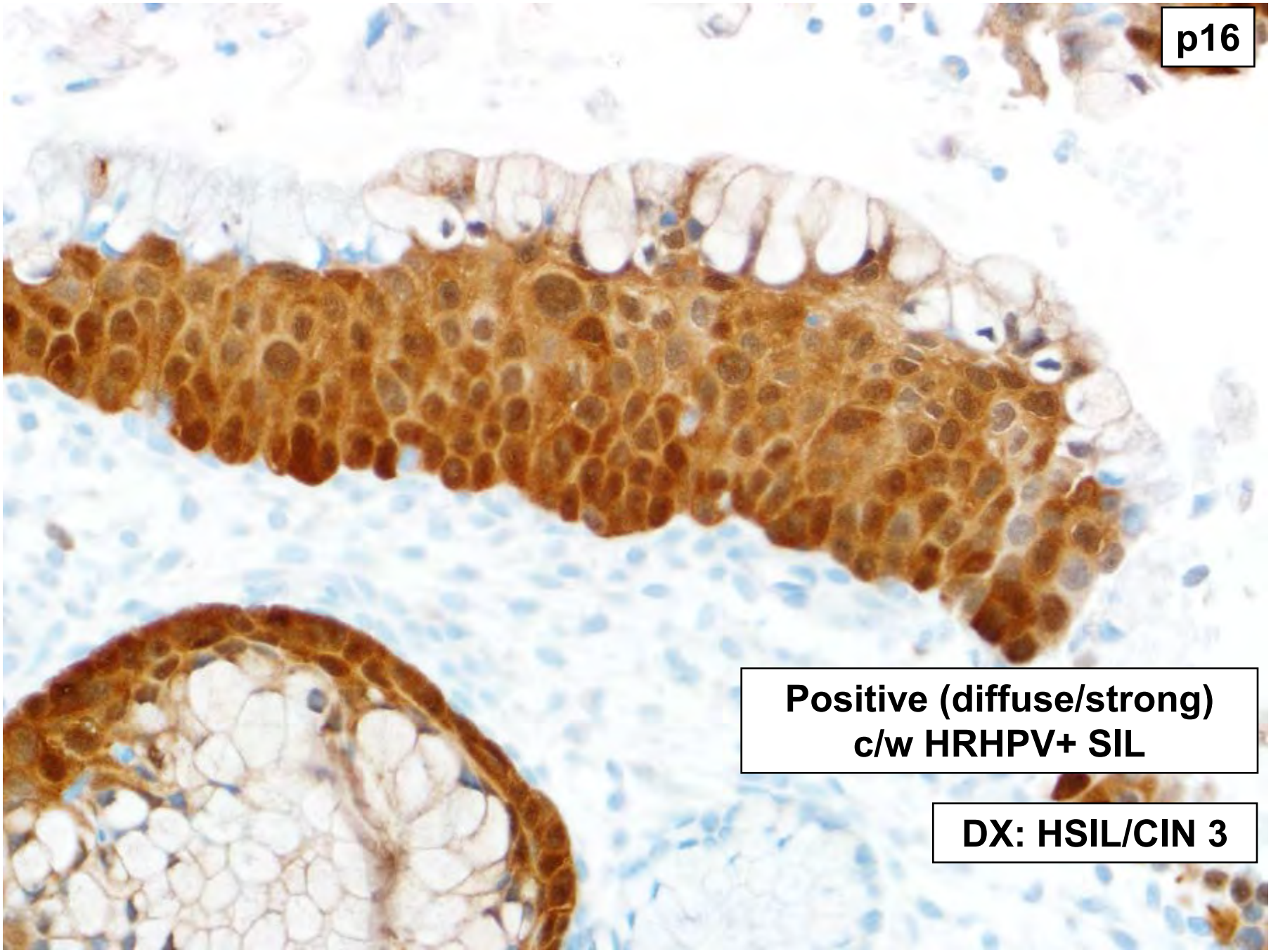

20 y.o.; NIL, HRHPV+ (Pap x2, 18 mo. apart);
AIM, ? HSIL
Mitotic figure

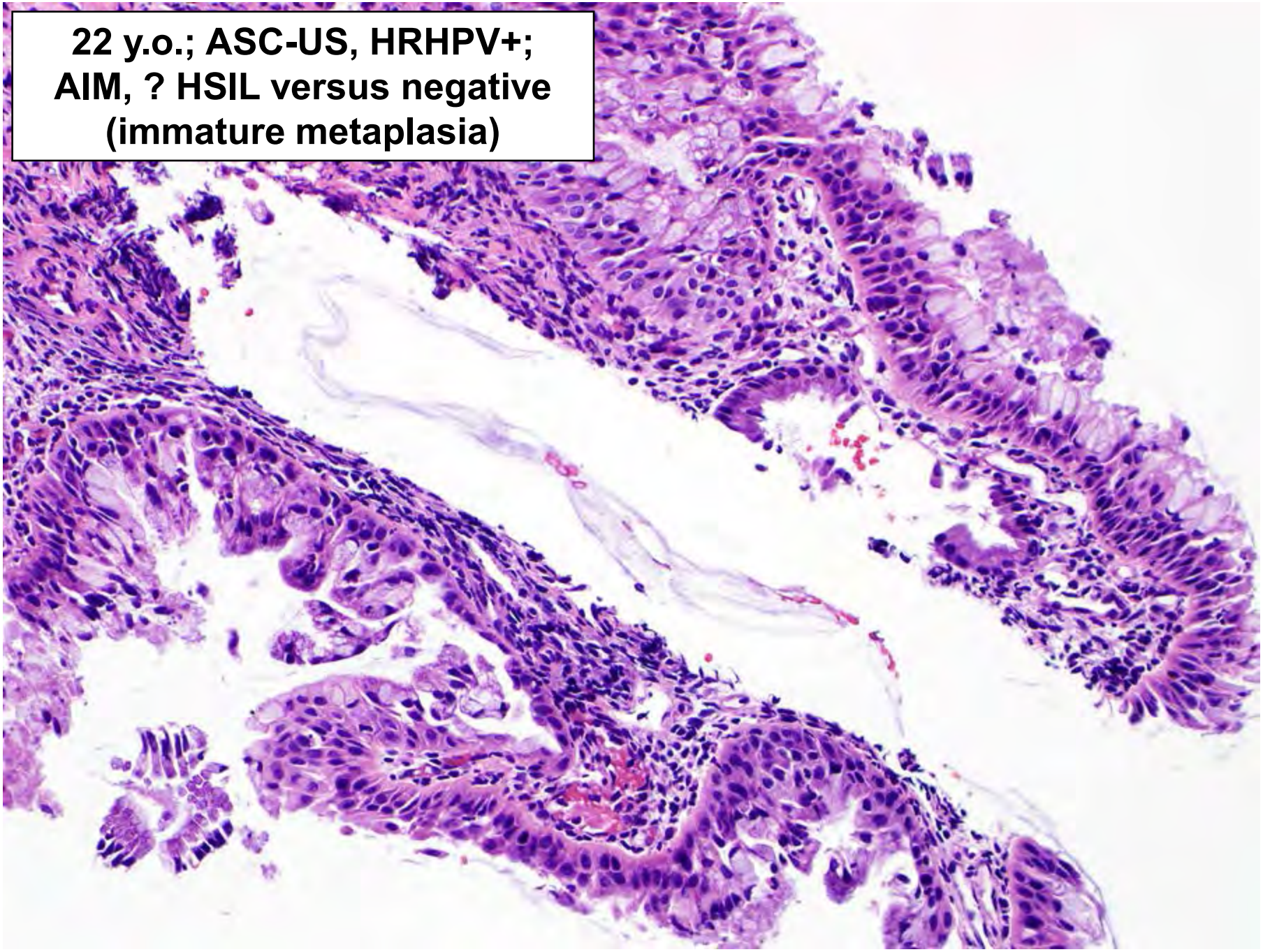

p16
Patchy pattern is not typical of HRHPV+ HSIL (repeat = same result)
DX: HSIL/CIN 2-3 (persistent HRHPV+, morphology)

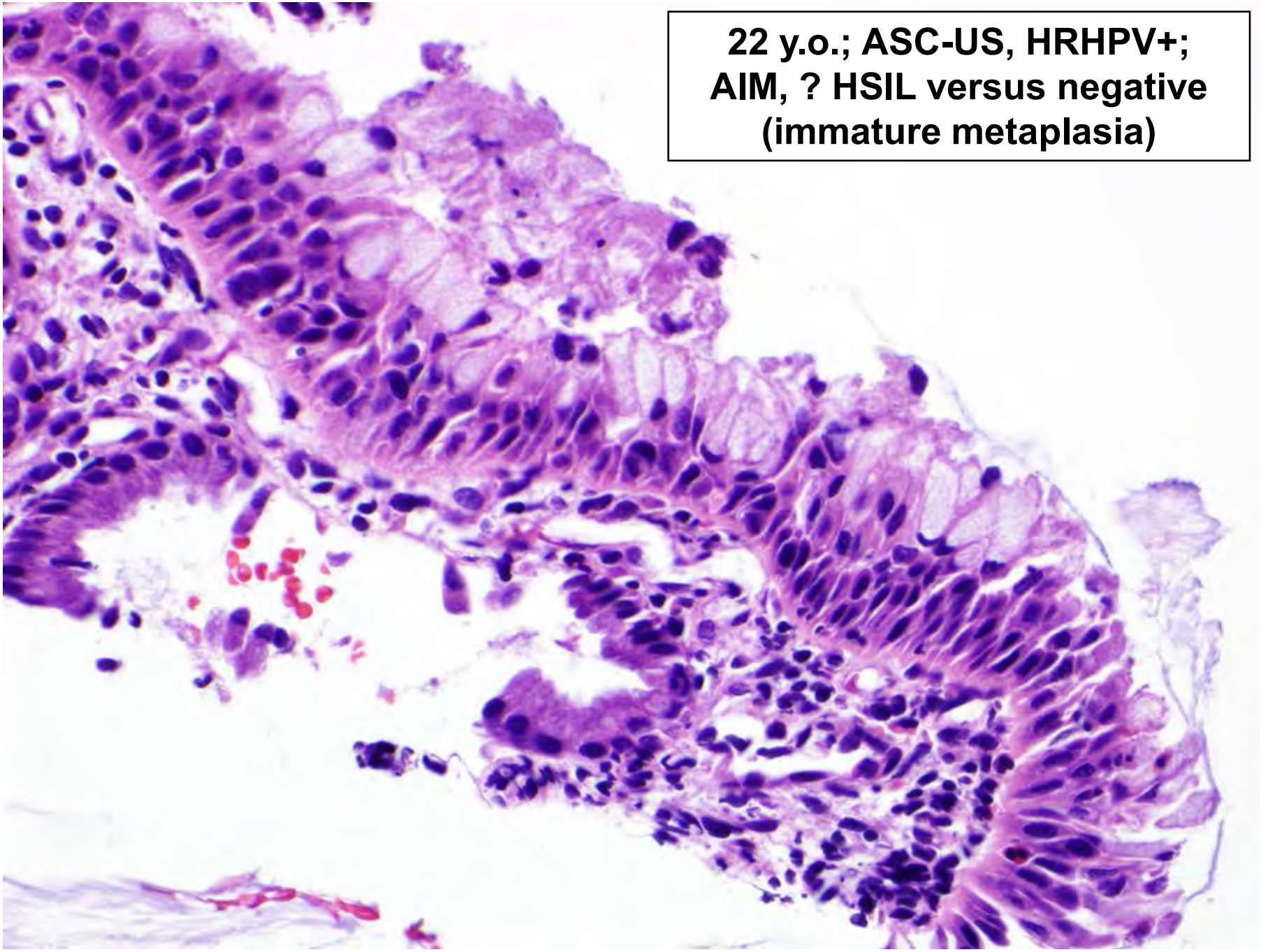

Ki-67
Increased in pattern c/w HSIL
(positive nuclei in all layers)
DX: HSIL/CIN 2-3
(HRHPV+, morphology, Ki-67 pattern)

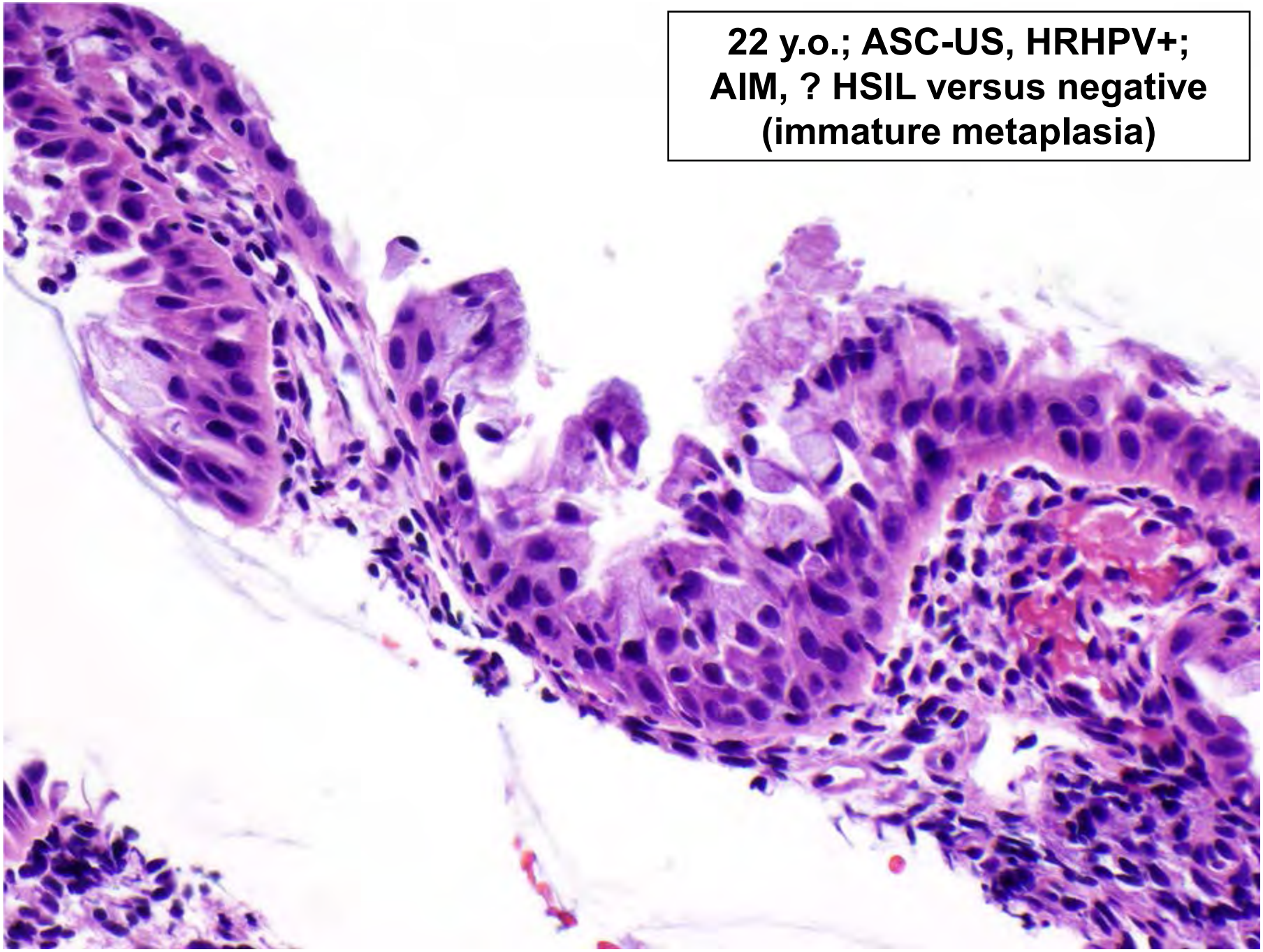

32 y.o.; ASC-H, HRHPV +;
AIM, ? HSIL versus negative
(immature metaplasia)

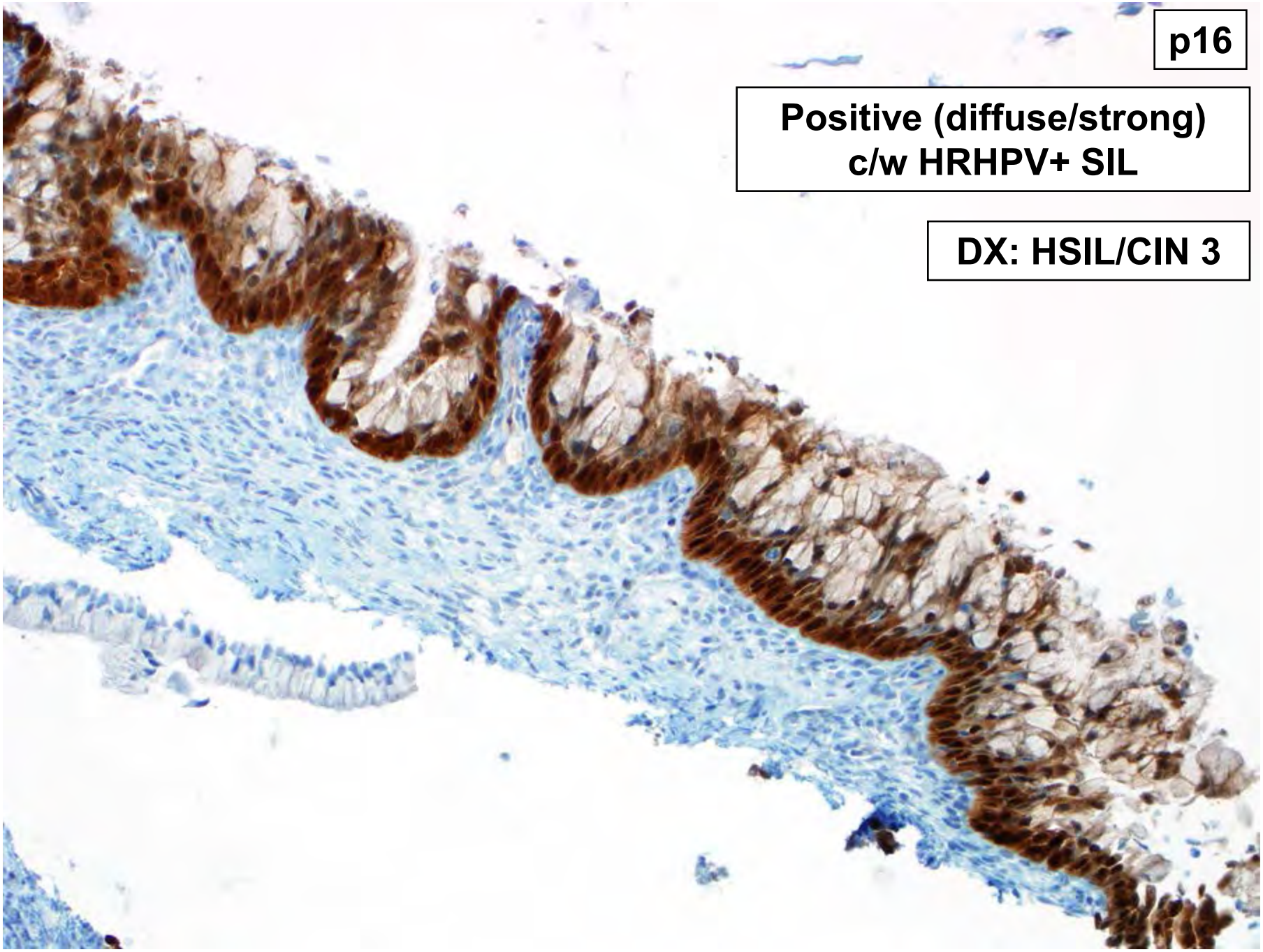

32 y.o.; ASC-H, HRHPV +;
AIM, ? HSIL versus negative
(immature metaplasia)

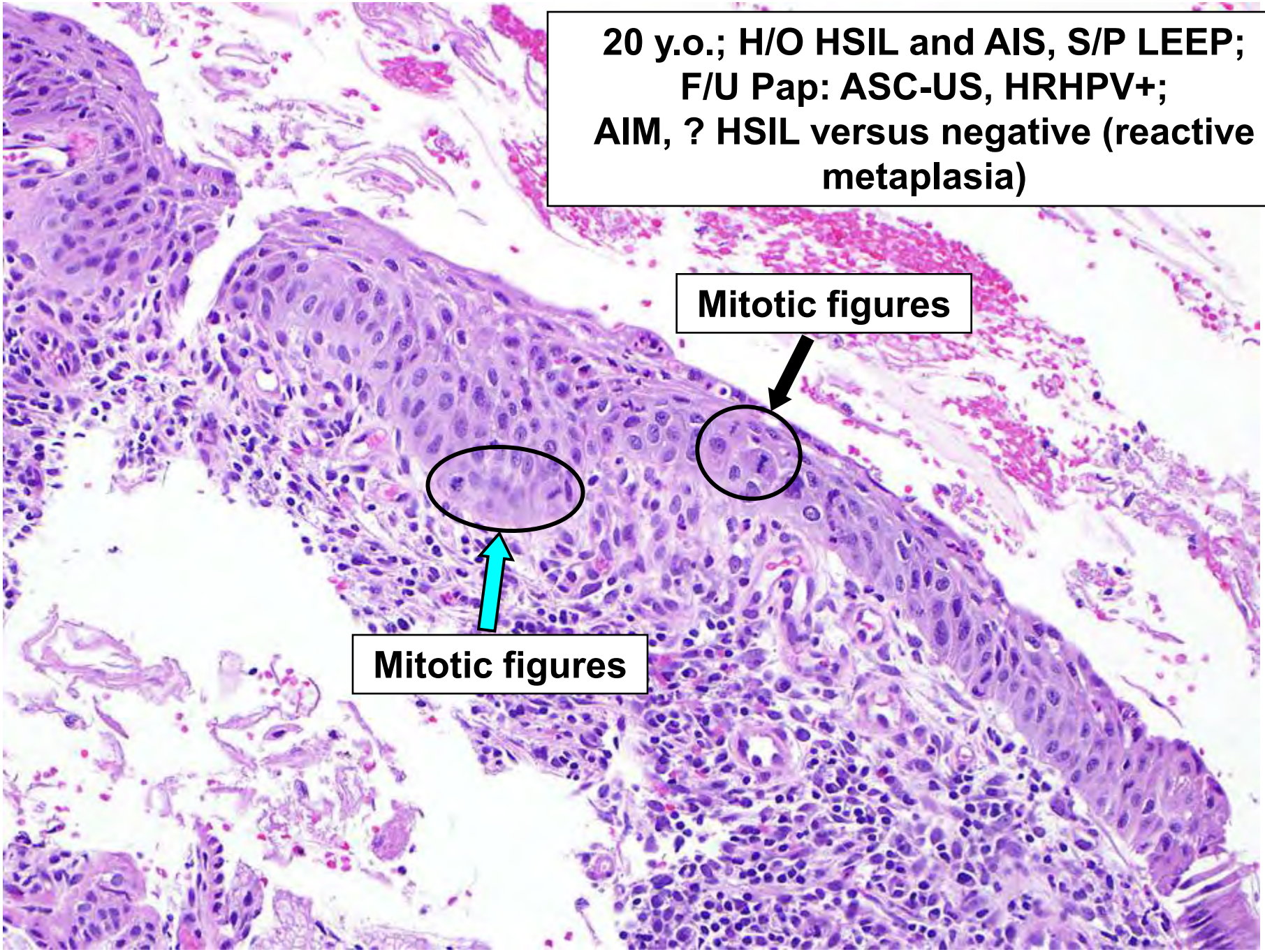

p16
Positive (diffuse/strong)
c/w HRHPV+ SIL
DX: HSIL/CIN 3

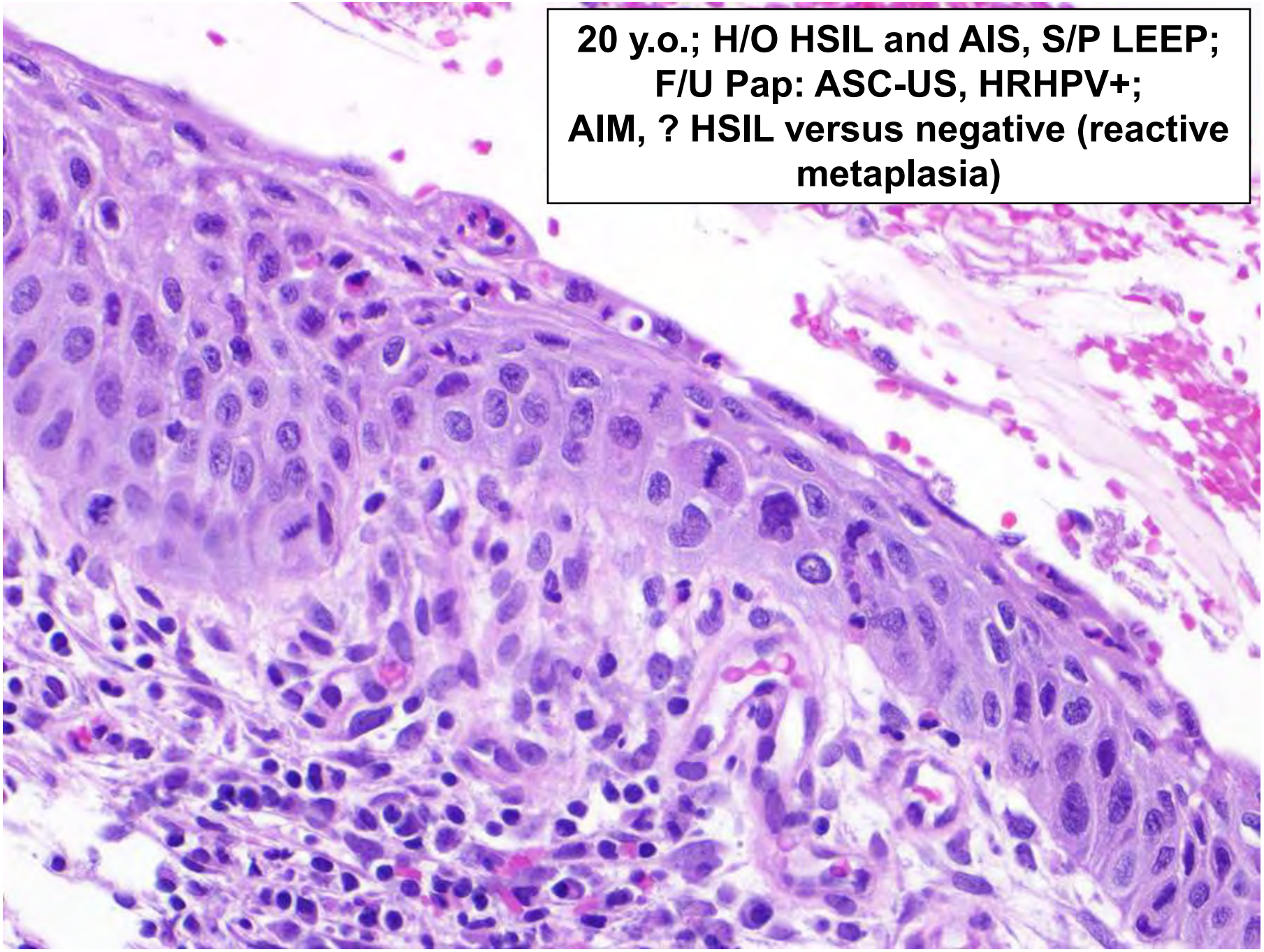

22 y.o.; ASC-US, HRHPV+;
AIM, ? HSIL versus negative
(immature metaplasia)

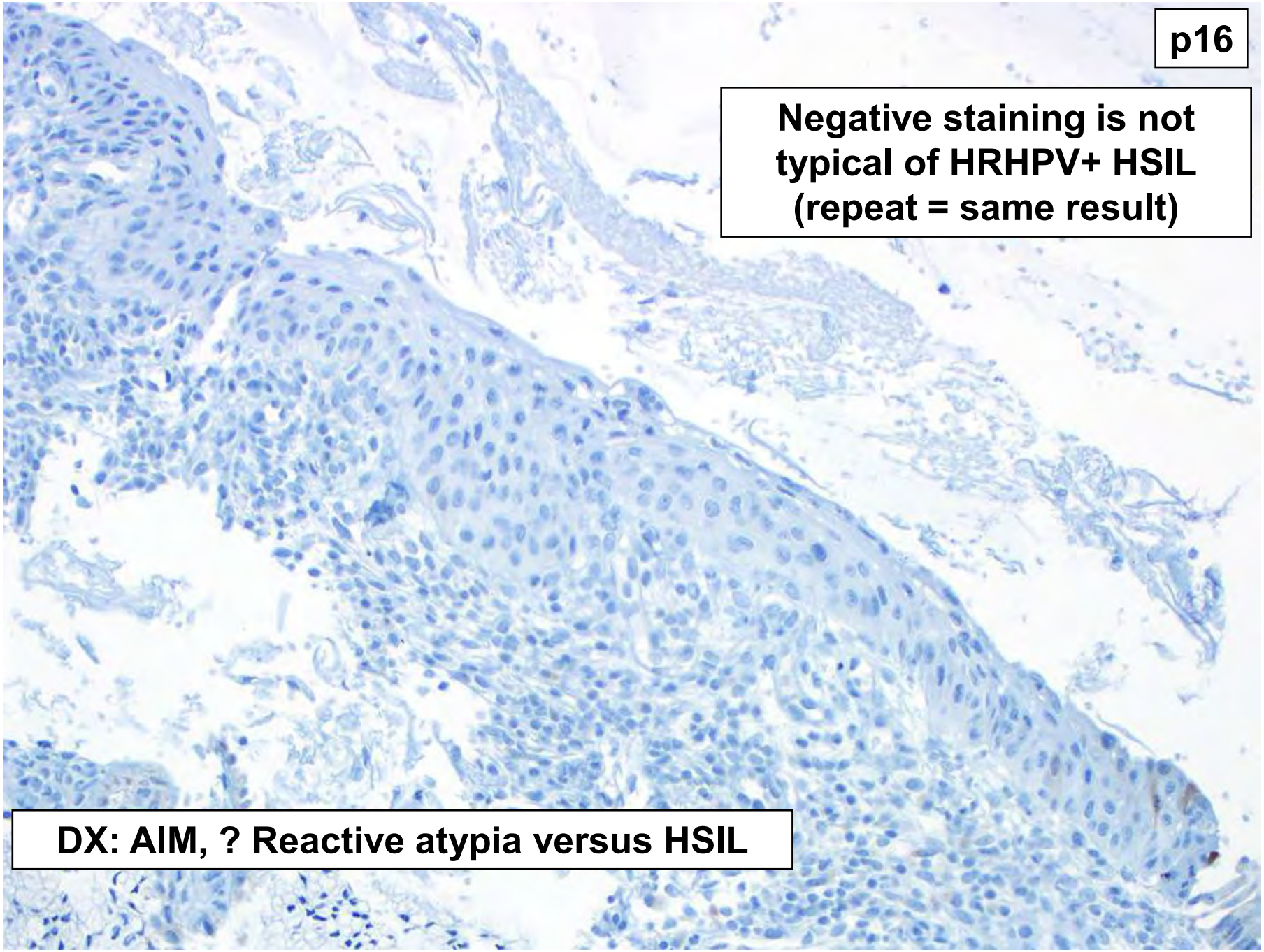

22 y.o.; ASC-US, HRHPV+;
AIM, ? HSIL versus negative
(immature metaplasia)

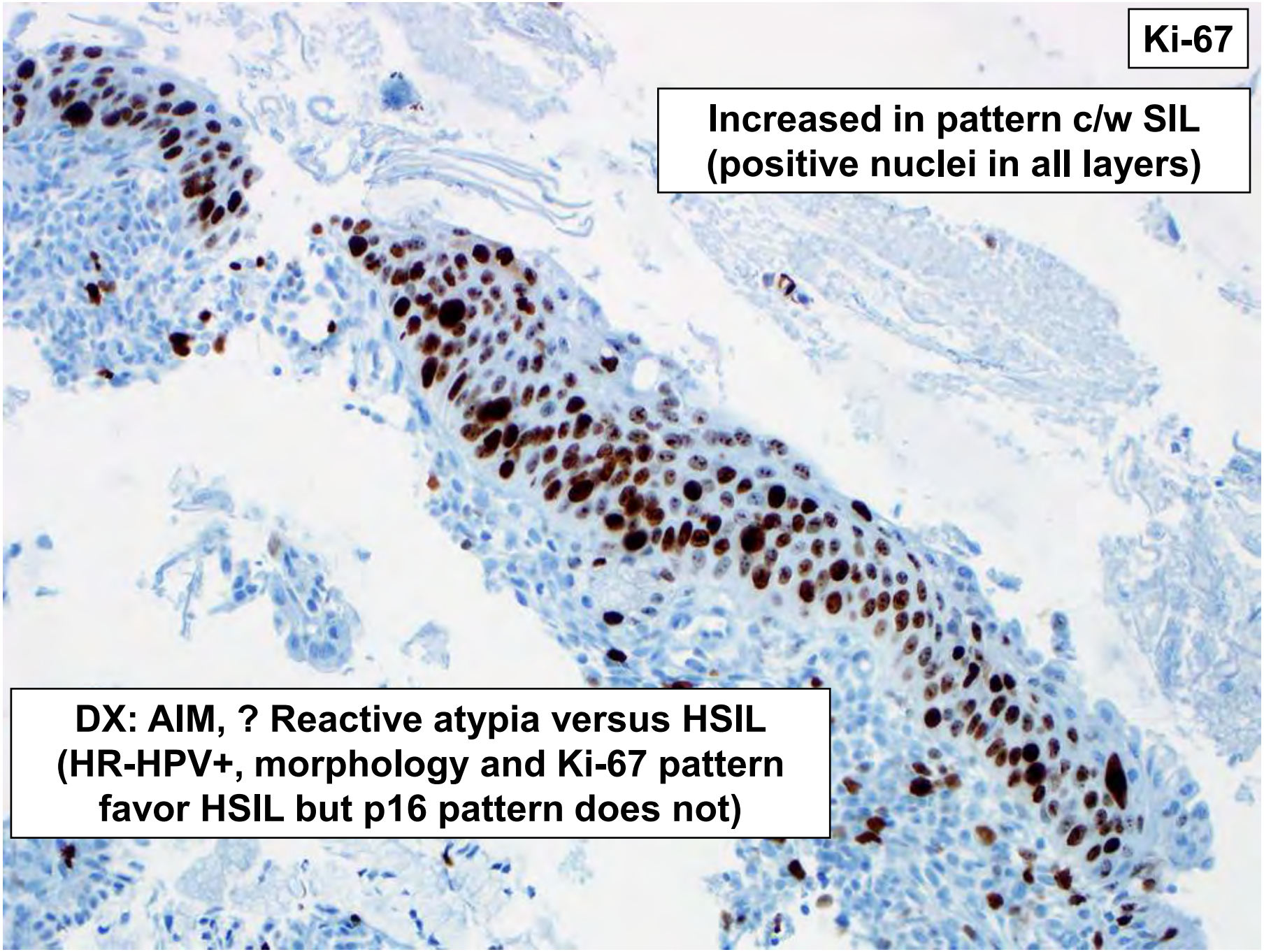

22 y.o.; ASC-US, HRHPV+;
AIM, ? HSIL versus negative
(immature metaplasia)

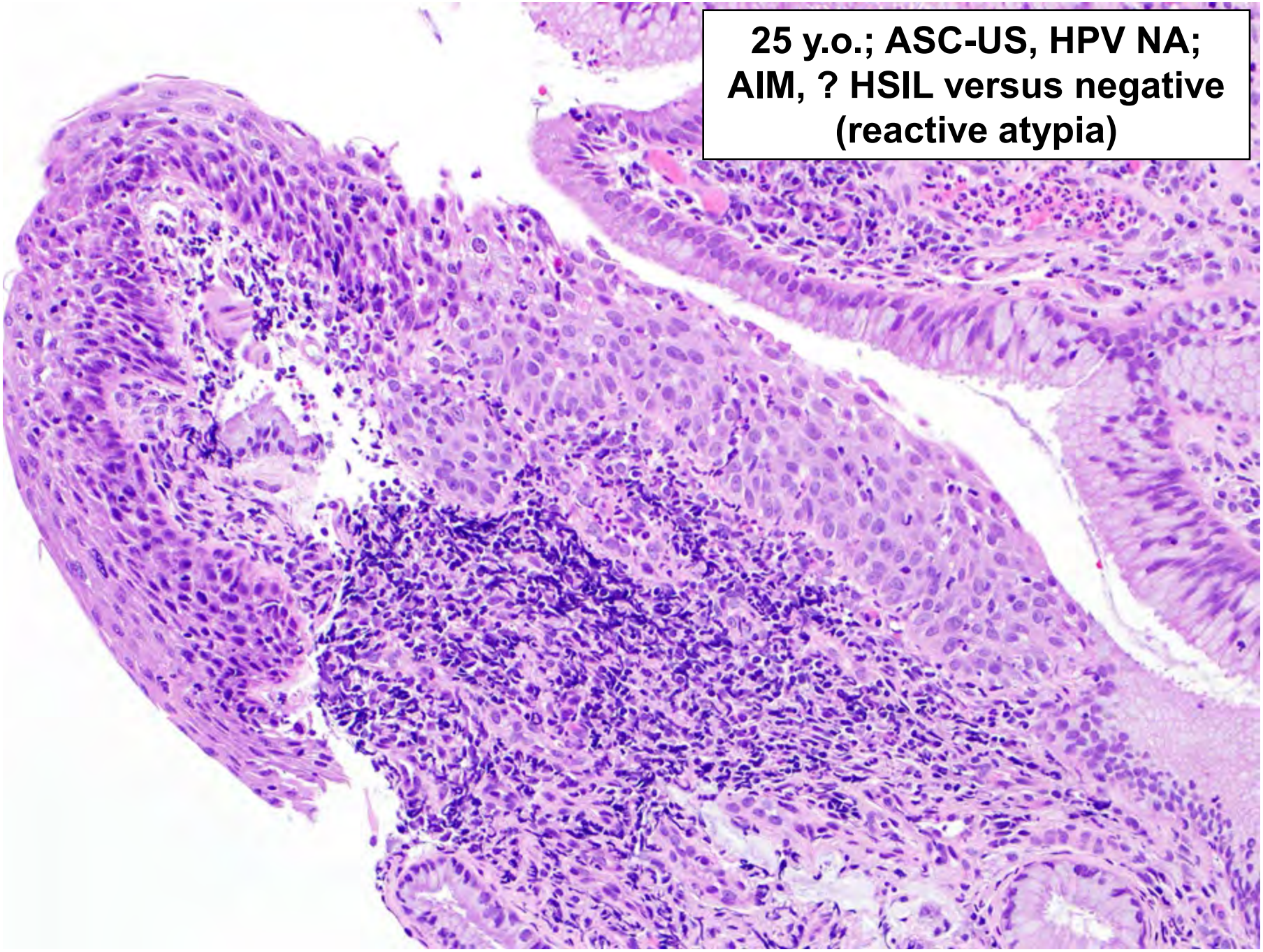

p16
Positive (diffuse/strong)
c/w HRHPV+ SIL
DX: HSIL/CIN 3

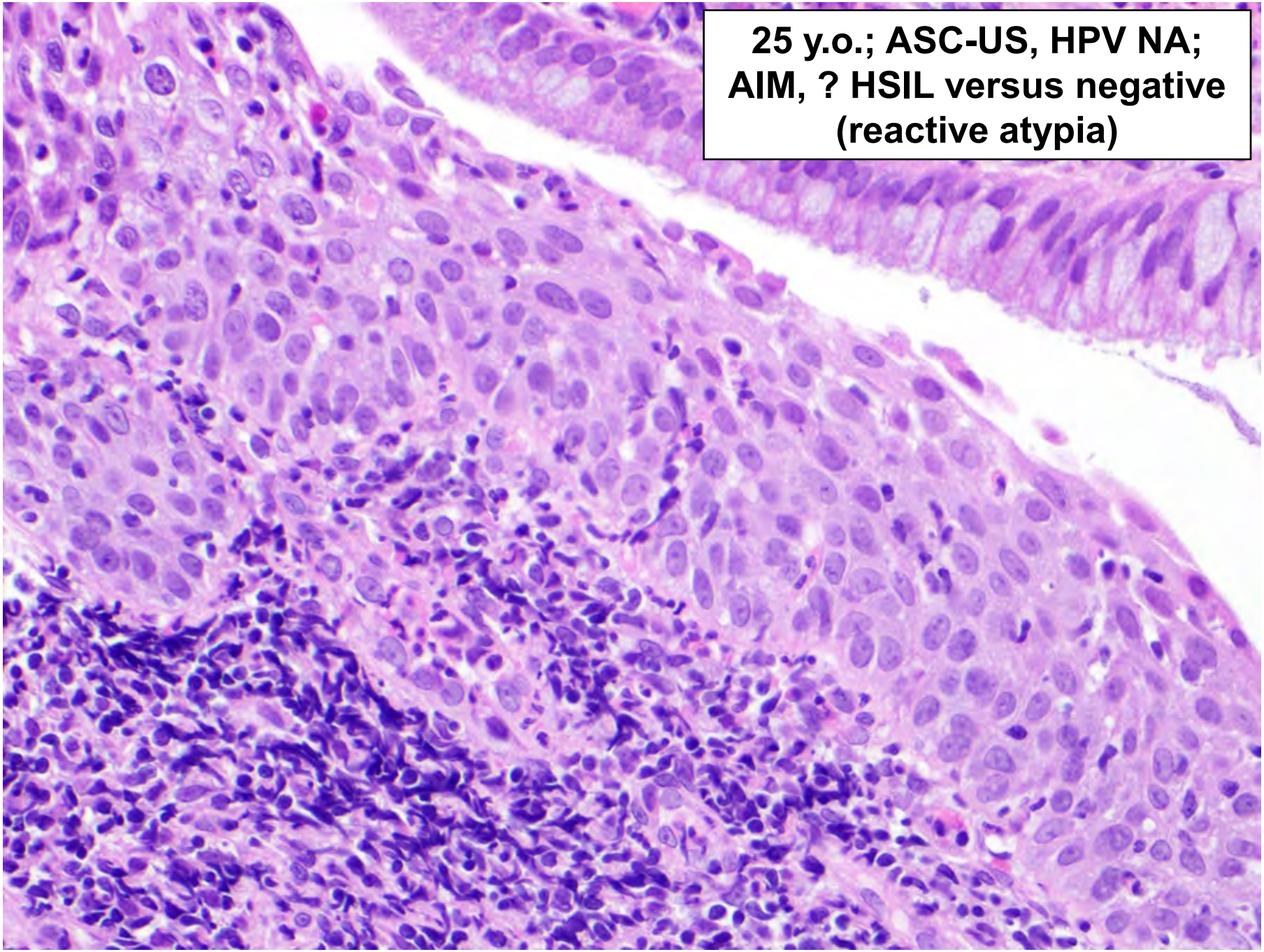

20 y.o.; H/O HSIL and AIS, S/P LEEP;
F/U Pap: ASC-US, HRHPV+;
AIM, ? HSIL versus negative (reactive metaplasia)
Mitotic figures
Mitotic figures

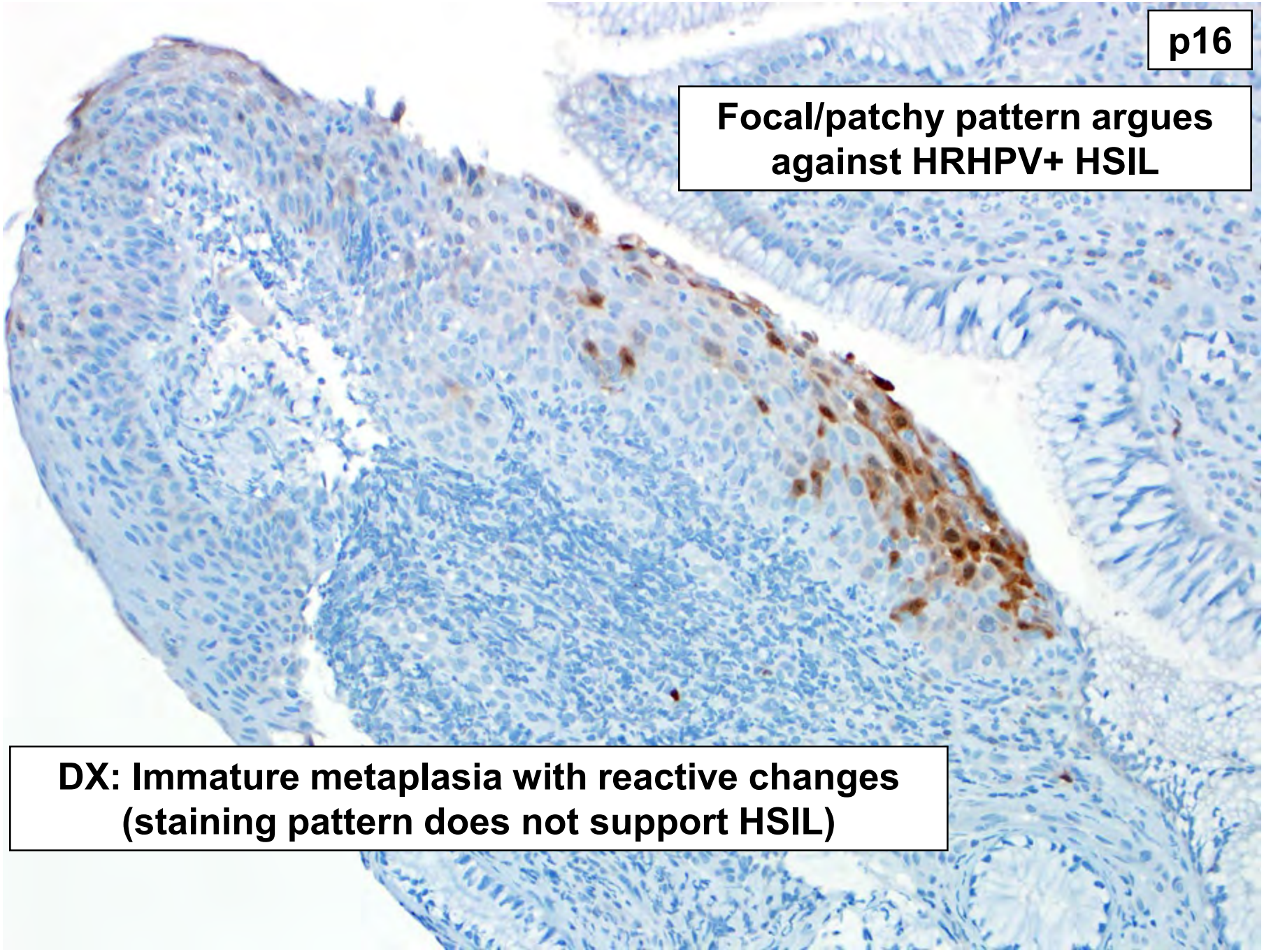

20 y.o.; H/O HSIL and AIS, S/P LEEP;
F/U Pap: ASC-US, HRHPV+;
AIM, ? HSIL versus negative (reactive metaplasia)

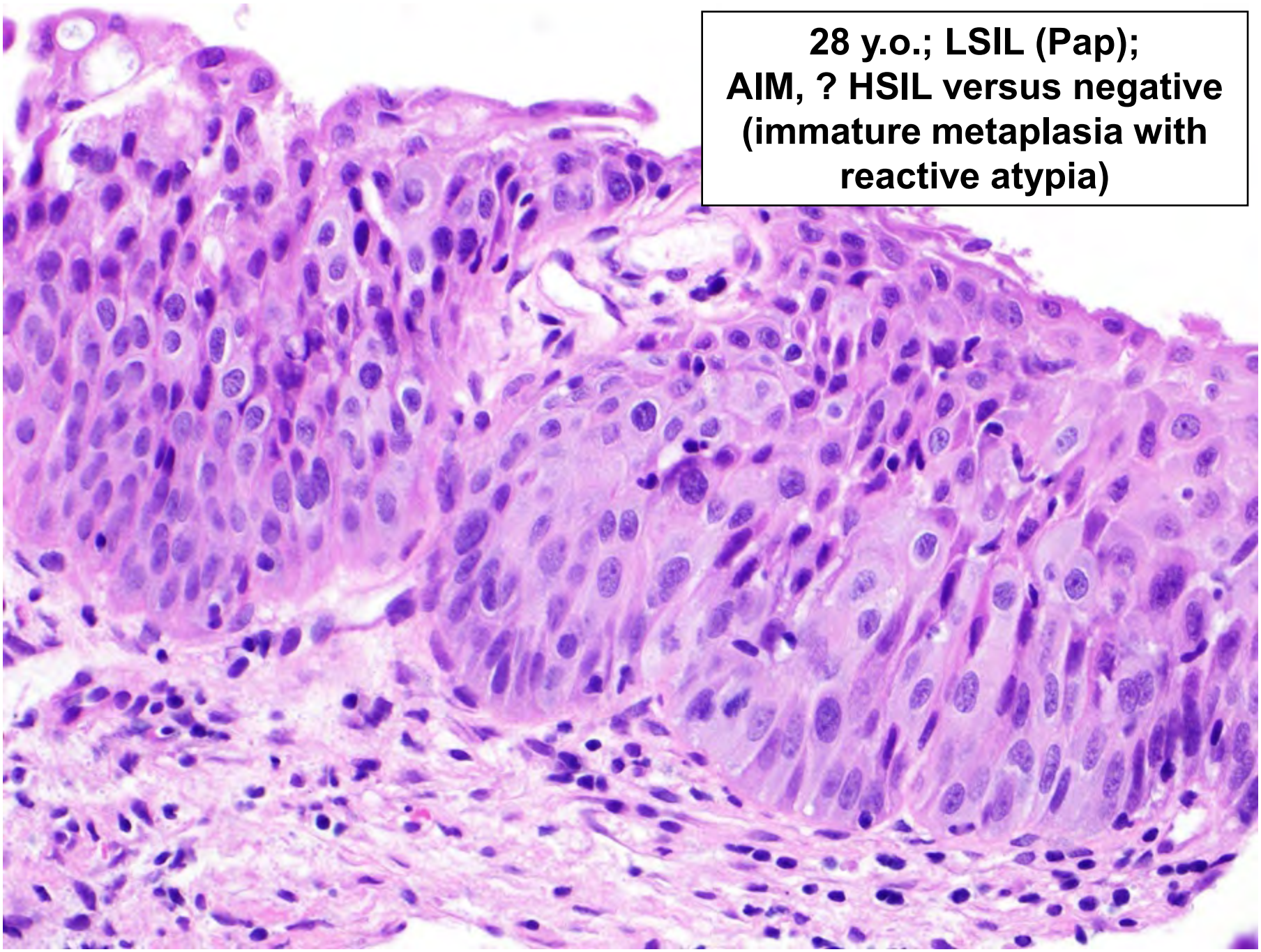

p16
Negative staining is not typical of HRHPV+ HSIL (repeat = same result)
DX: AIM, ? Reactive atypia versus HSIL

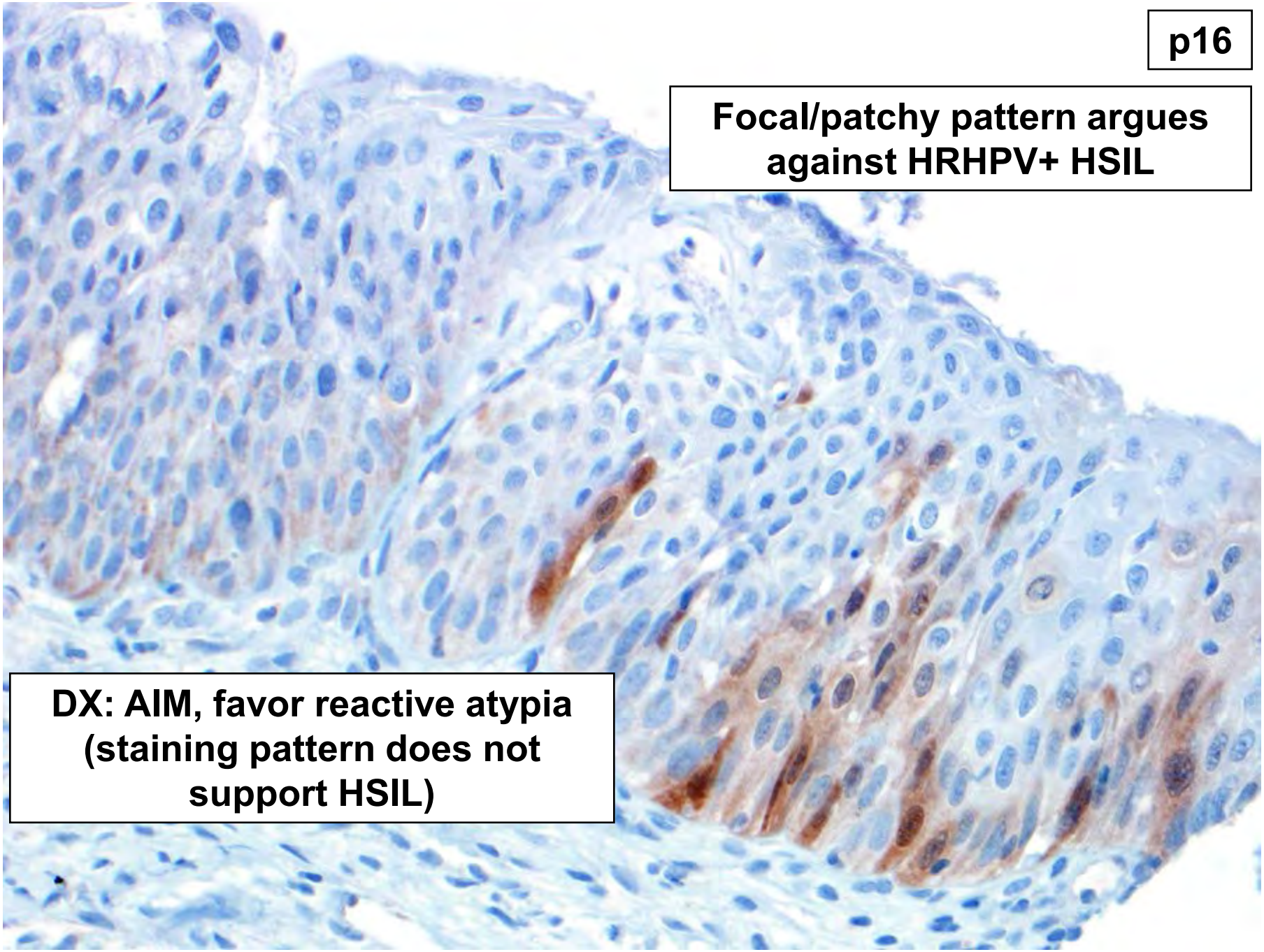

Ki-67
Increased in pattern c/w SIL
(positive nuclei in all layers)
DX: AIM, ? Reactive atypia versus HSIL
(HR-HPV+, morphology and Ki-67 pattern
favor HSIL but p16 pattern does not)

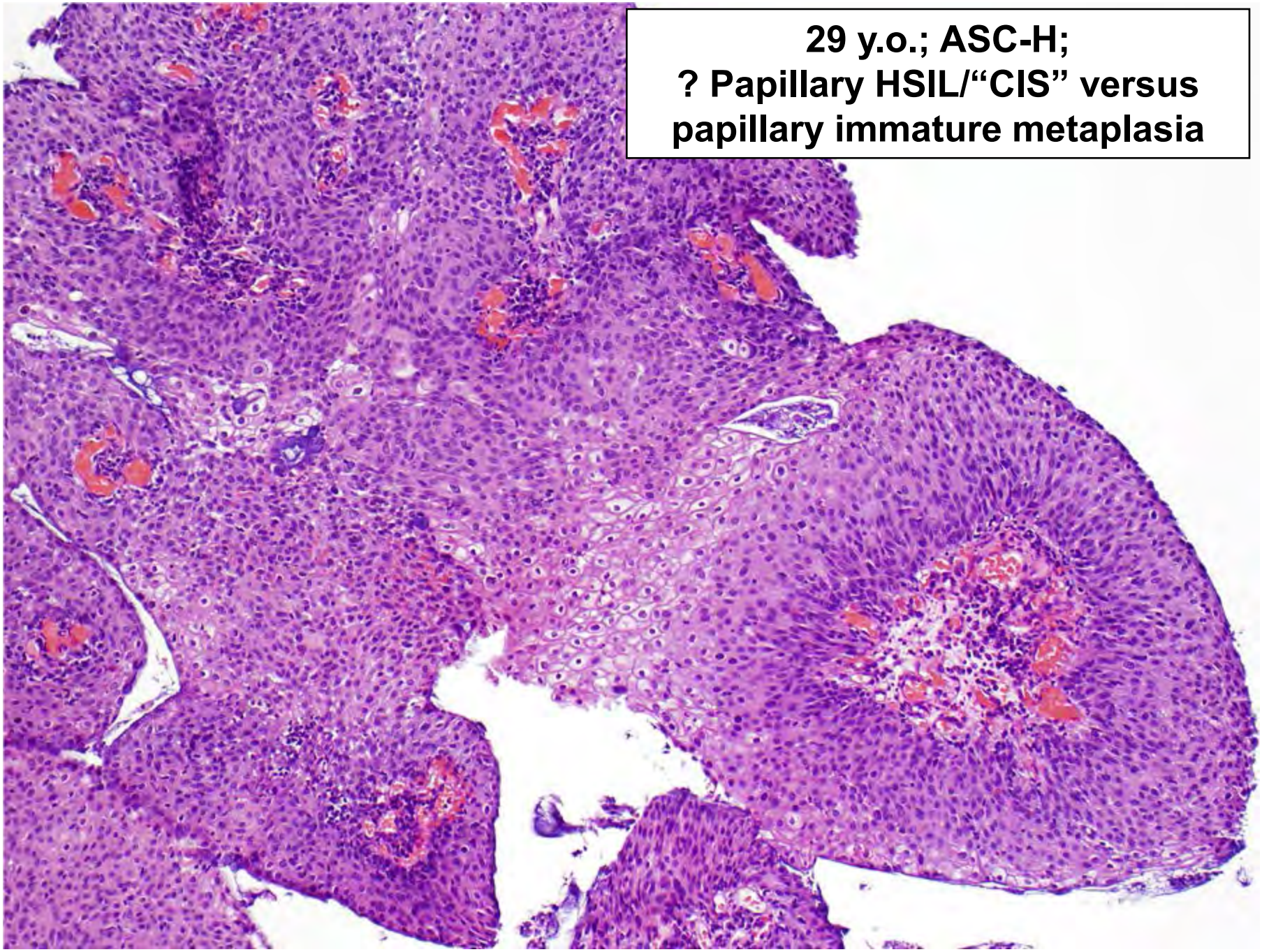

25 y.o.; ASC-US, HPV NA;
AIM, ? HSIL versus negative
(reactive atypia)

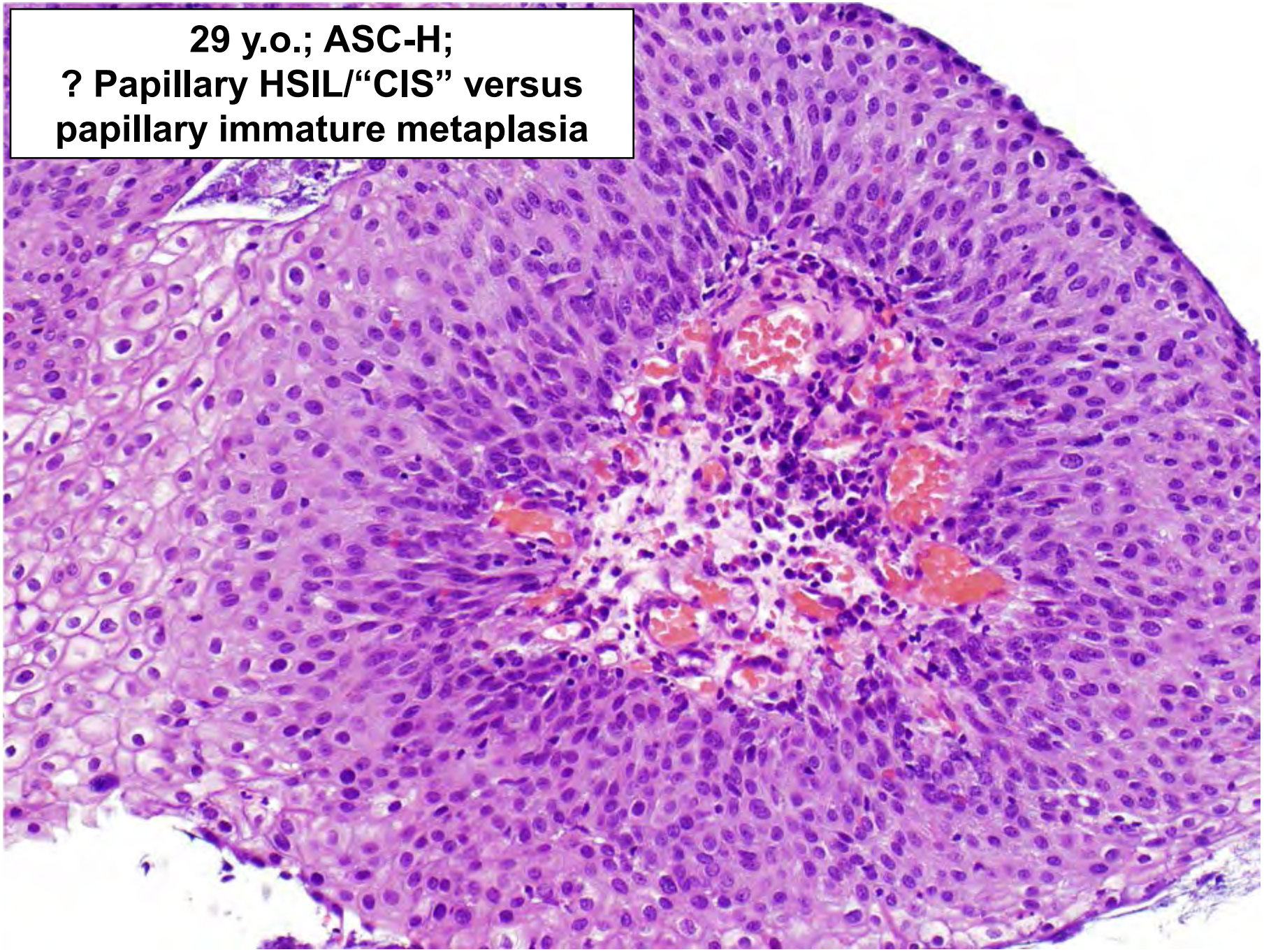

25 y.o.; ASC-US, HPV NA;
AIM, ? HSIL versus negative
(reactive atypia)

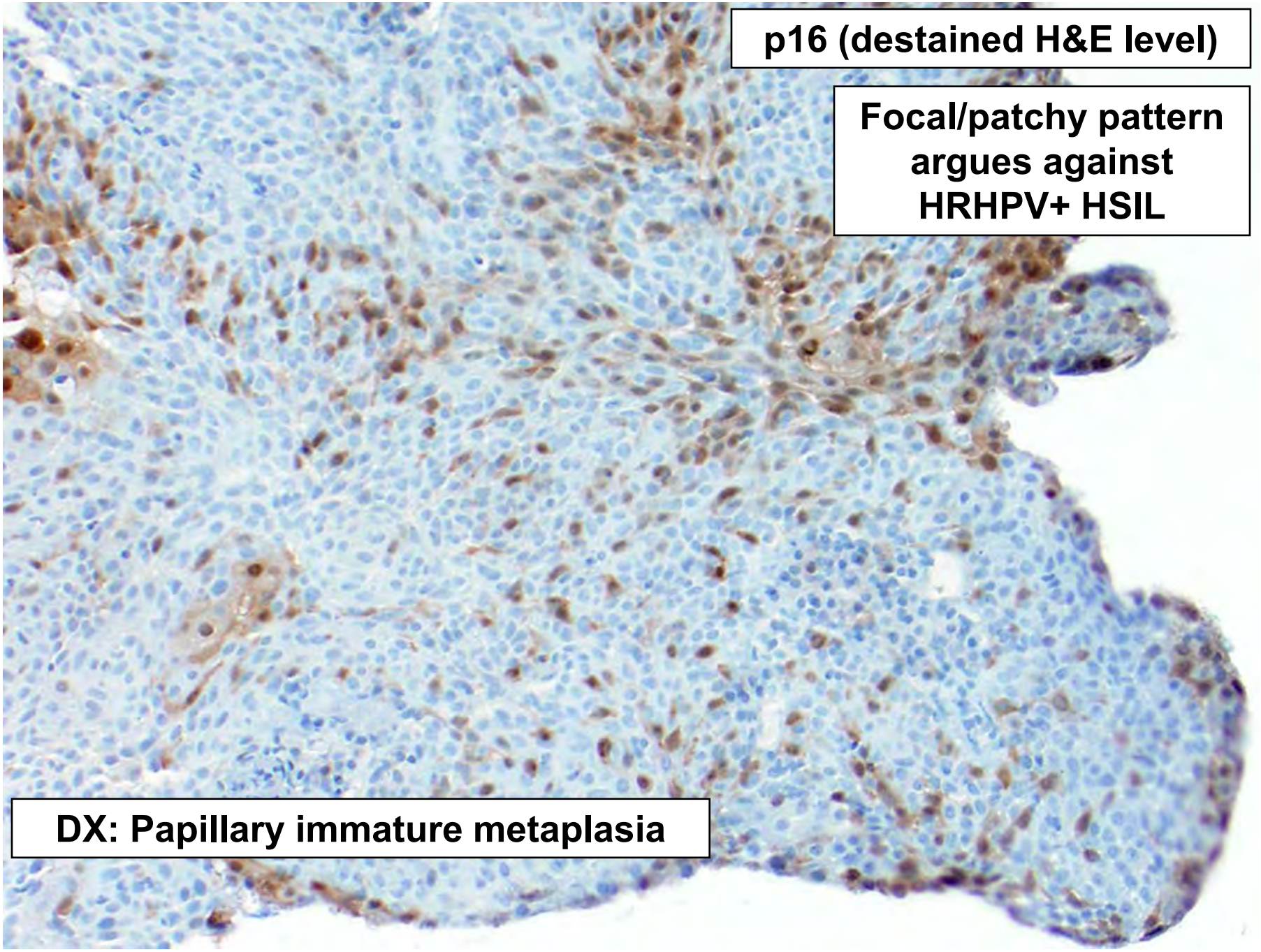

p16
Focal/patchy pattern argues against HRHPV+ HSIL
DX: Immature metaplasia with reactive changes (staining pattern does not support HSIL)

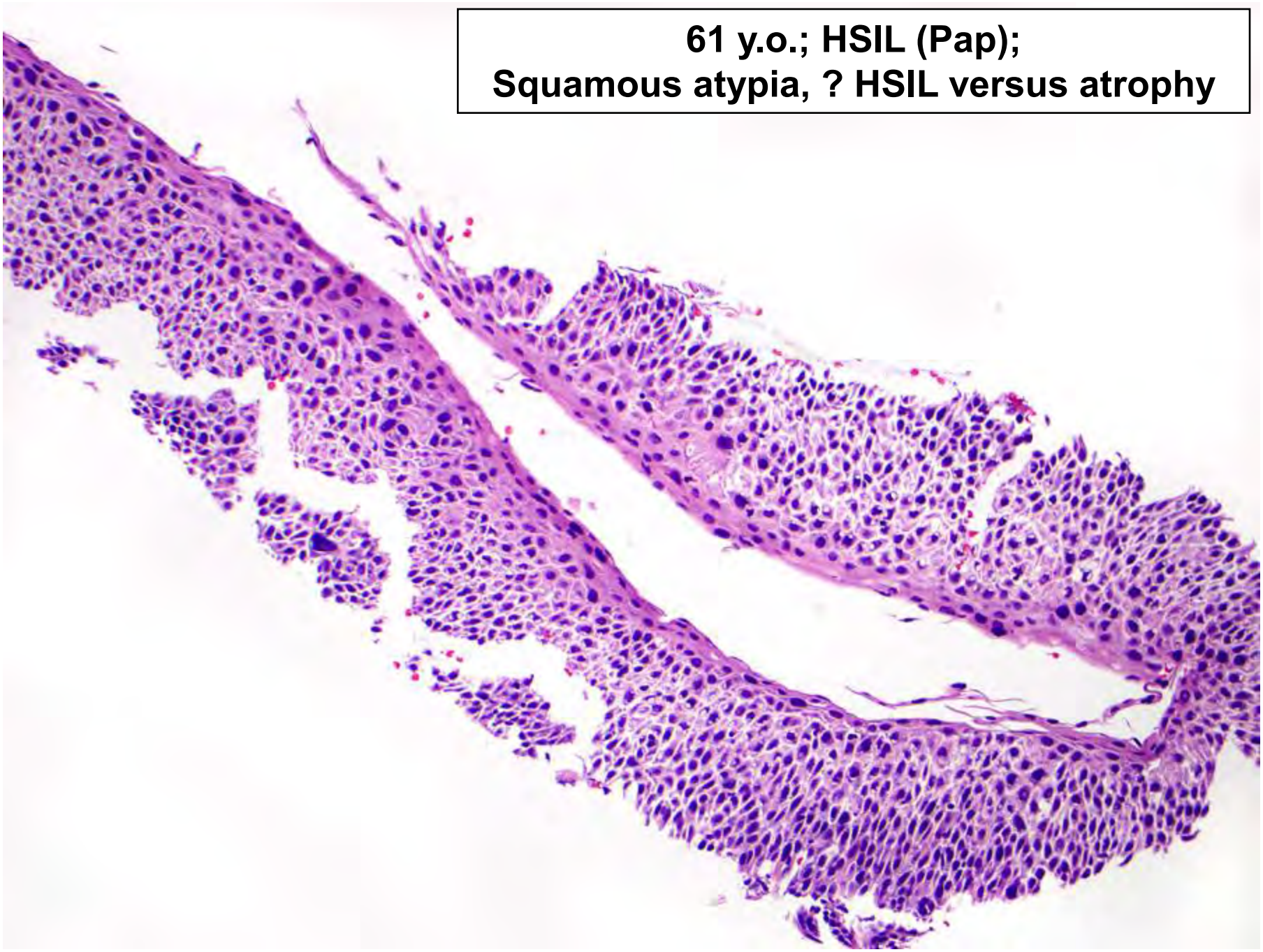

28 y.o.; LSIL (Pap);
AIM, ? HSIL versus negative
(immature metaplasia with
reactive atypia)

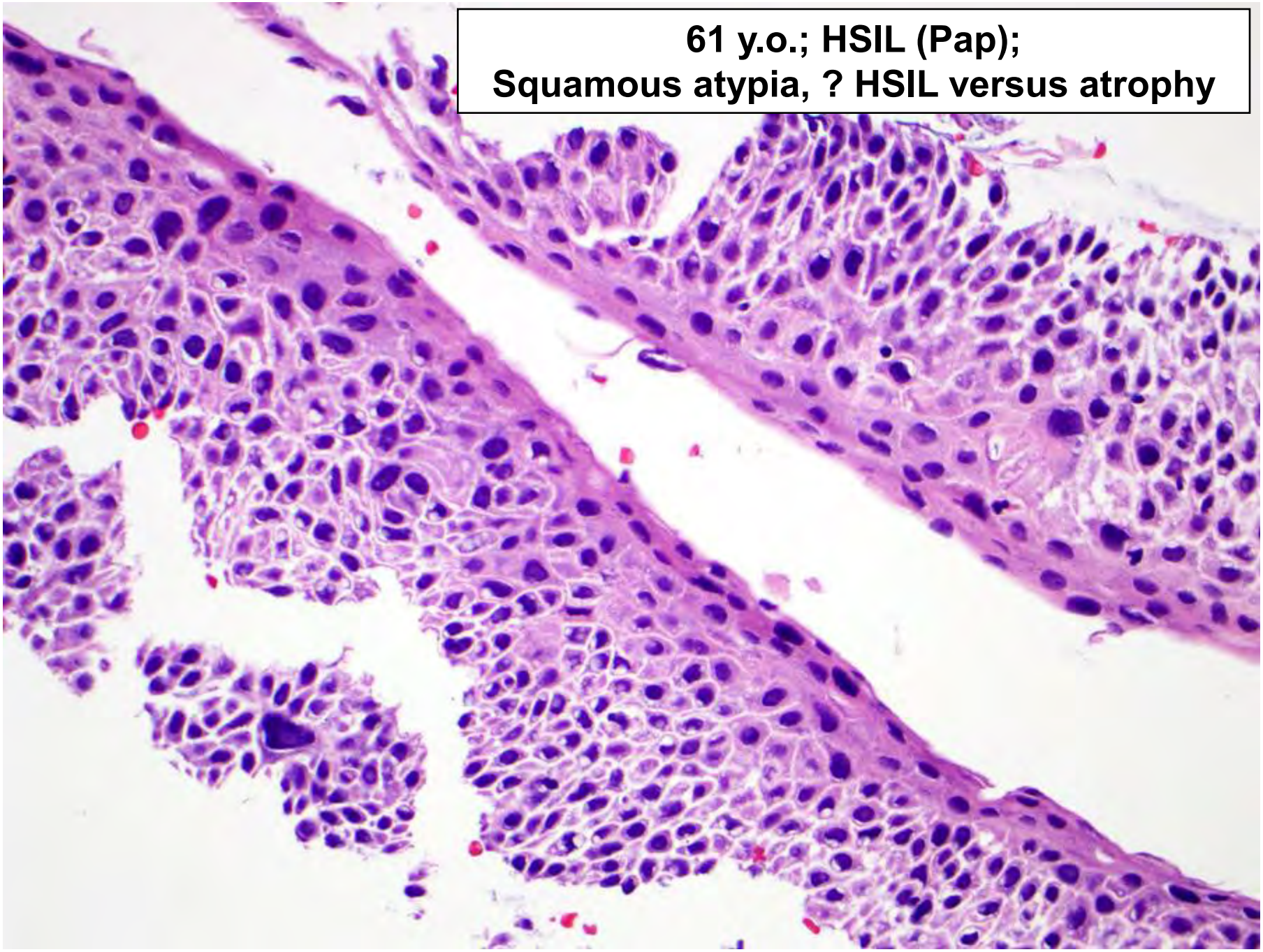

p16
Focal/patchy pattern argues against HRHPV+ HSIL
DX: AIM, favor reactive atypia (staining pattern does not support HSIL)

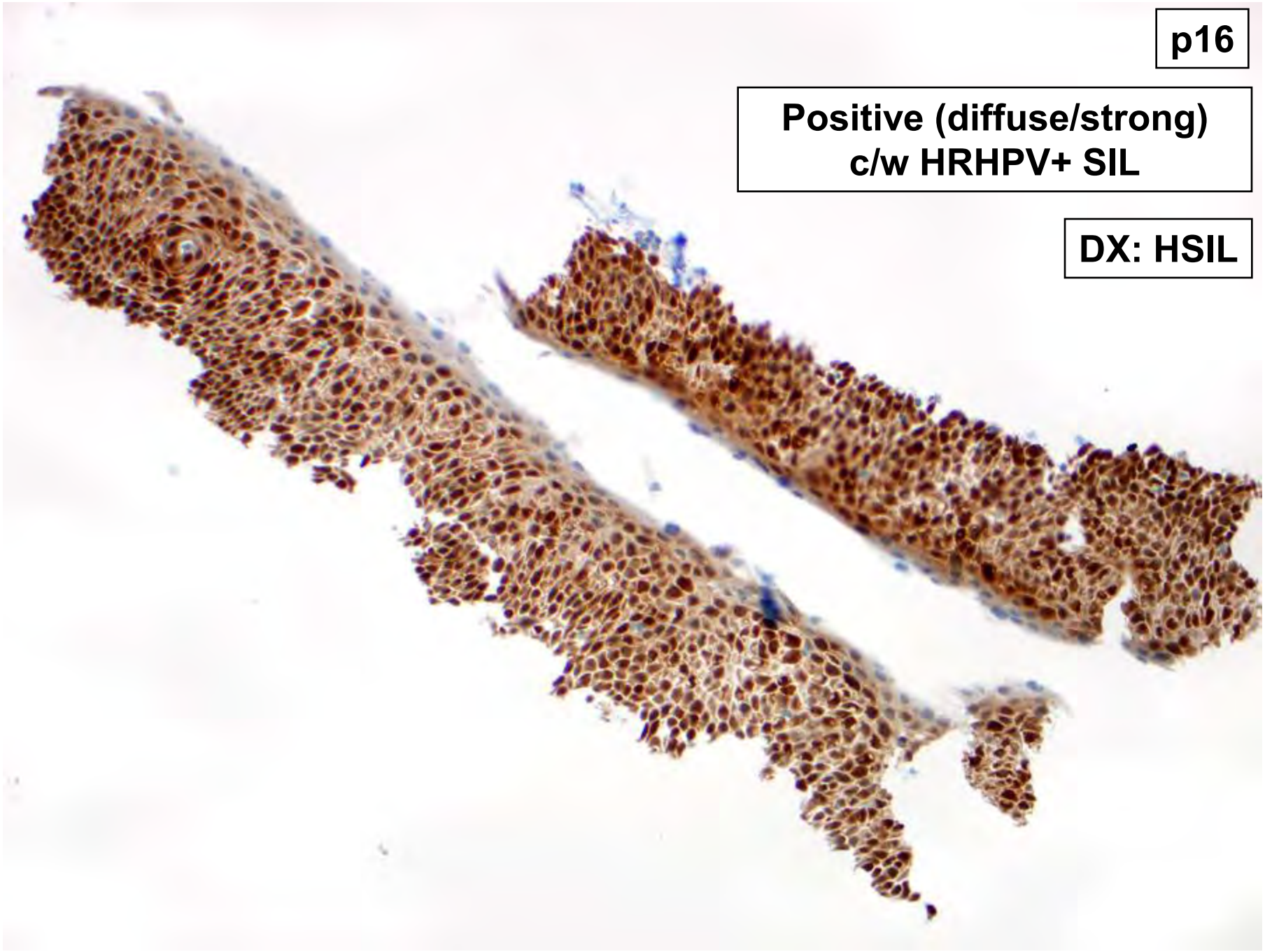

29 y.o.; ASC-H;
? Papillary HSIL/“CIS” versus
papillary immature metaplasia

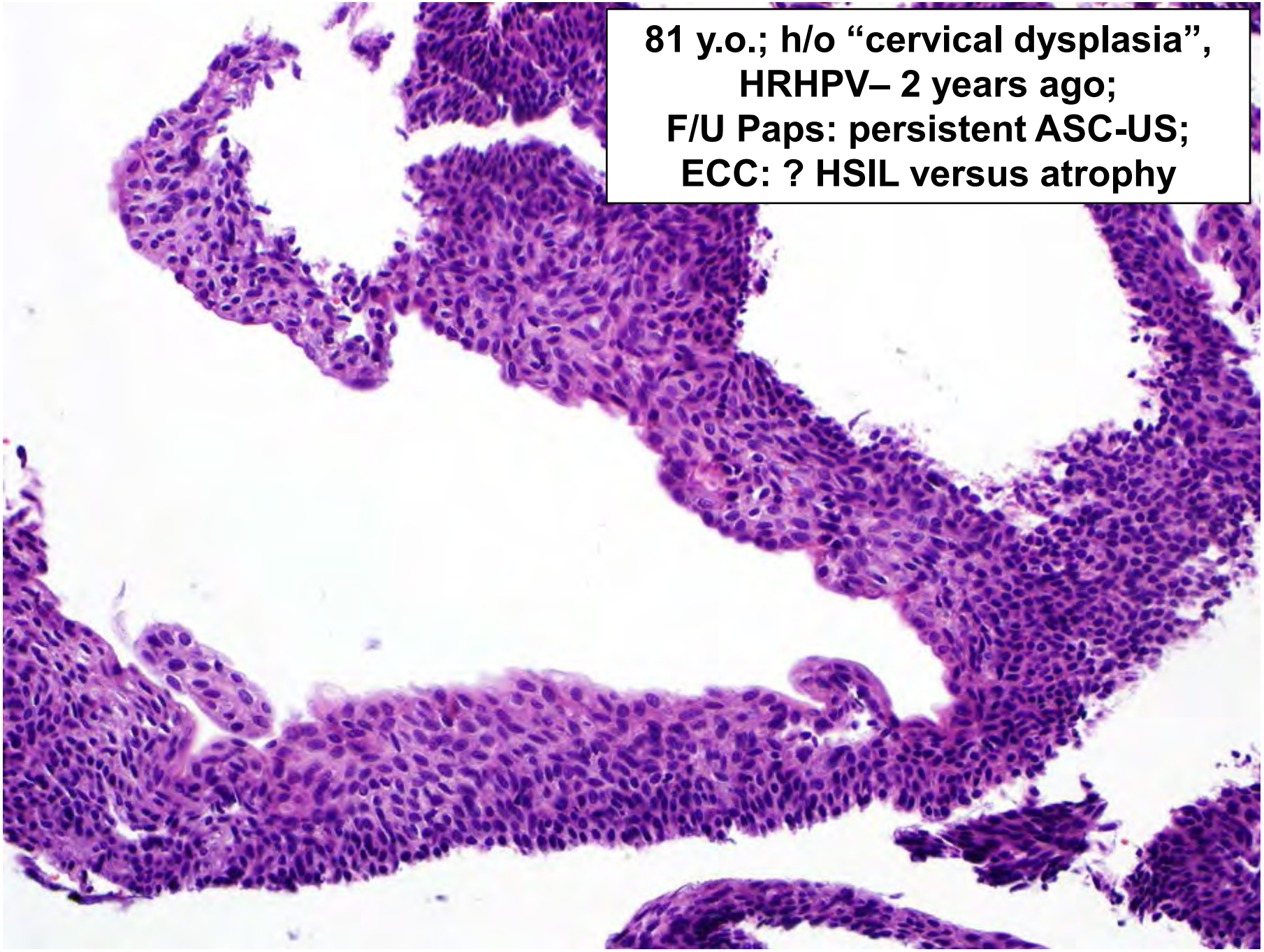

29 y.o.; ASC-H;
? Papillary HSIL/“CIS” versus
papillary immature metaplasia

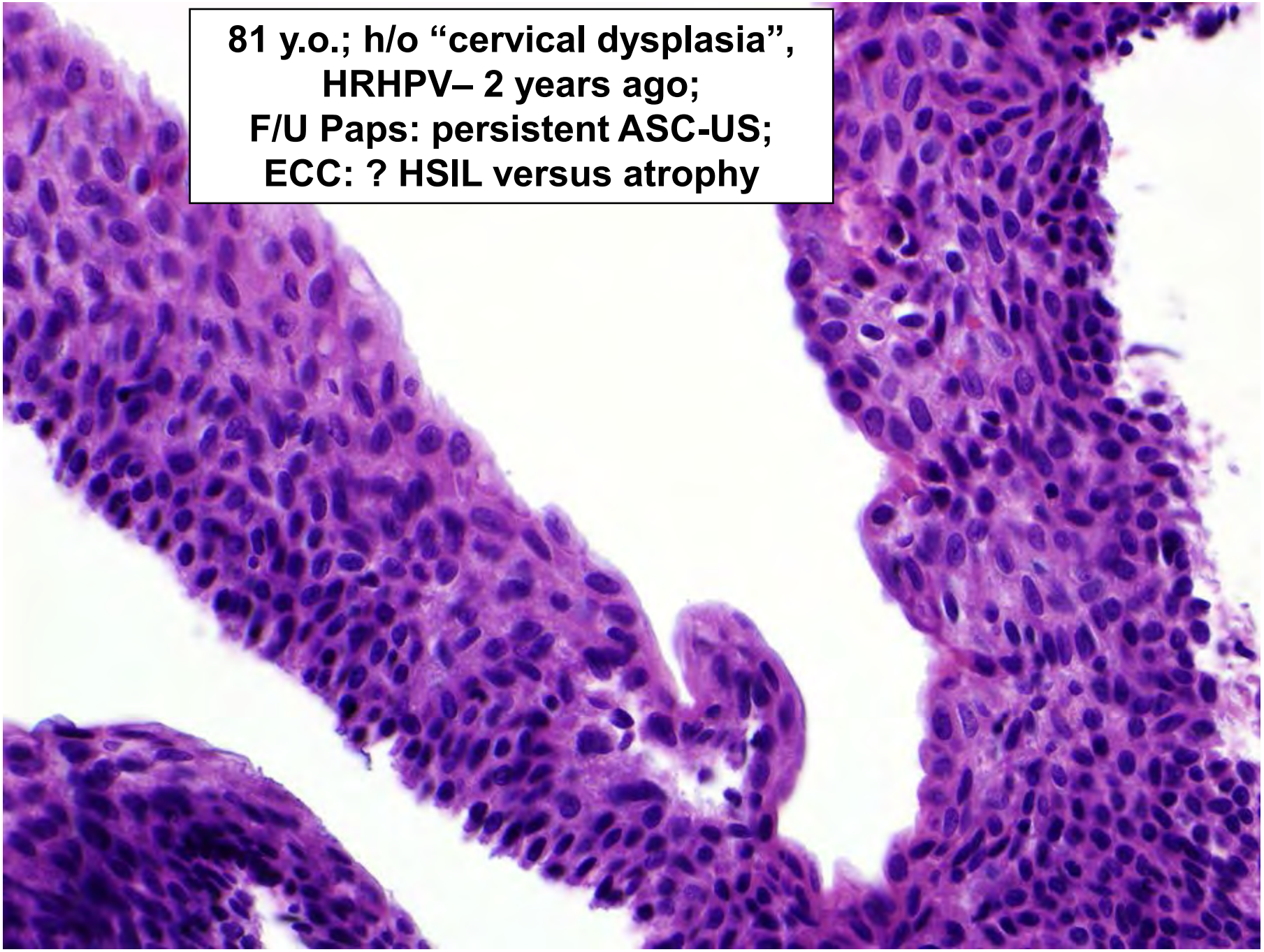

p16 (destained H&E level)
Focal/patchy pattern argues against HRHPV+ HSIL
DX: Papillary immature metaplasia

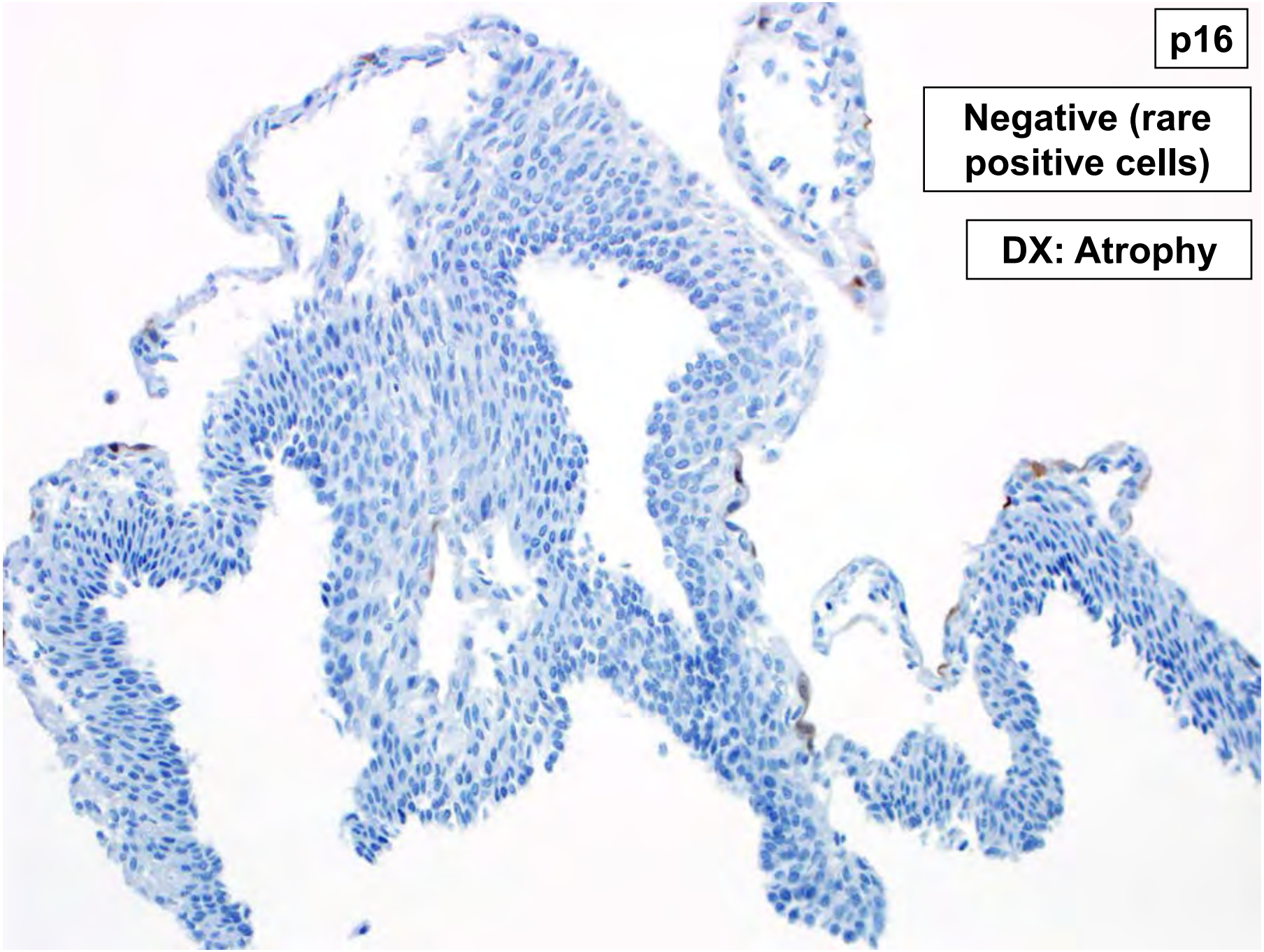

61 y.o.; HSIL (Pap);
Squamous atypia, ? HSIL versus atrophy


61 y.o.; HSIL (Pap);
Squamous atypia, ? HSIL versus atrophy

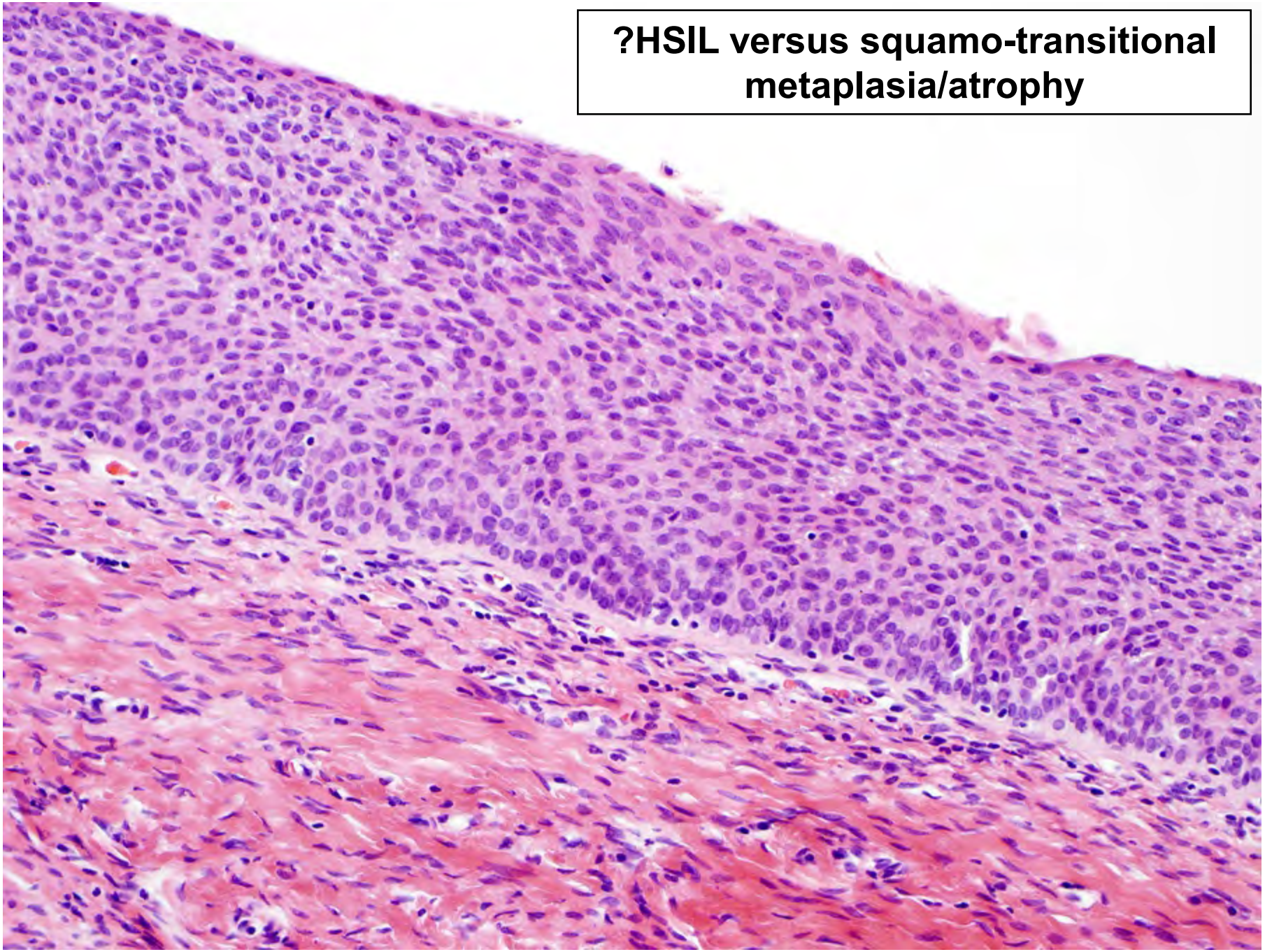

p16
Positive (diffuse/strong)
c/w HRHPV+ SIL
DX: HSIL


81 y.o.; h/o “cervical dysplasia”,
HRHPV– 2 years ago;
F/U Paps: persistent ASC-US;
ECC: ? HSIL versus atrophy


81 y.o.; h/o “cervical dysplasia”,
HRHPV– 2 years ago;
F/U Paps: persistent ASC-US;
ECC: ? HSIL versus atrophy

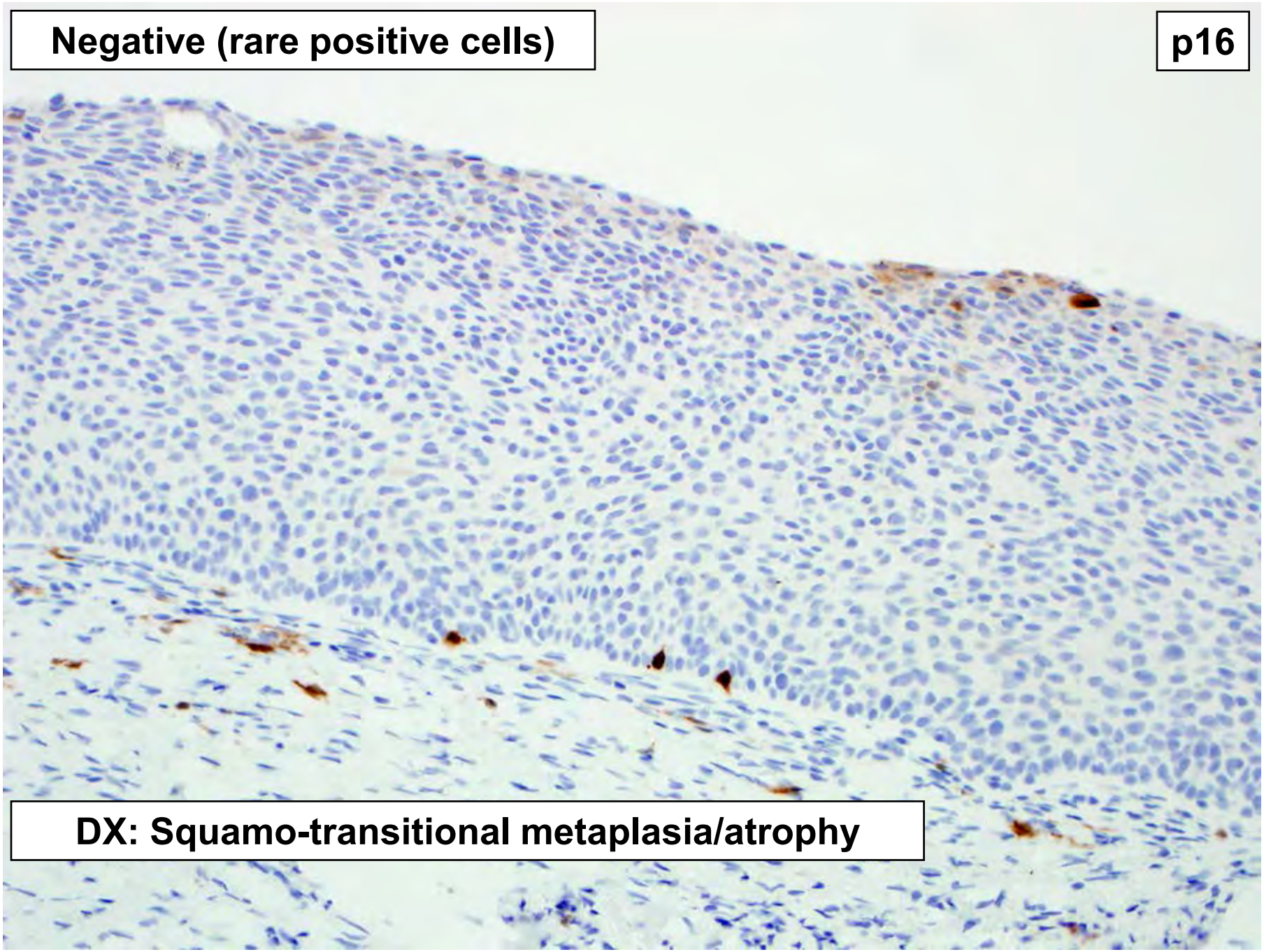

p16
Negative (rare positive cells)
DX: Atrophy

?HSIL versus squamo-transitional metaplasia/atrophy



Negative (rare positive cells)

p16

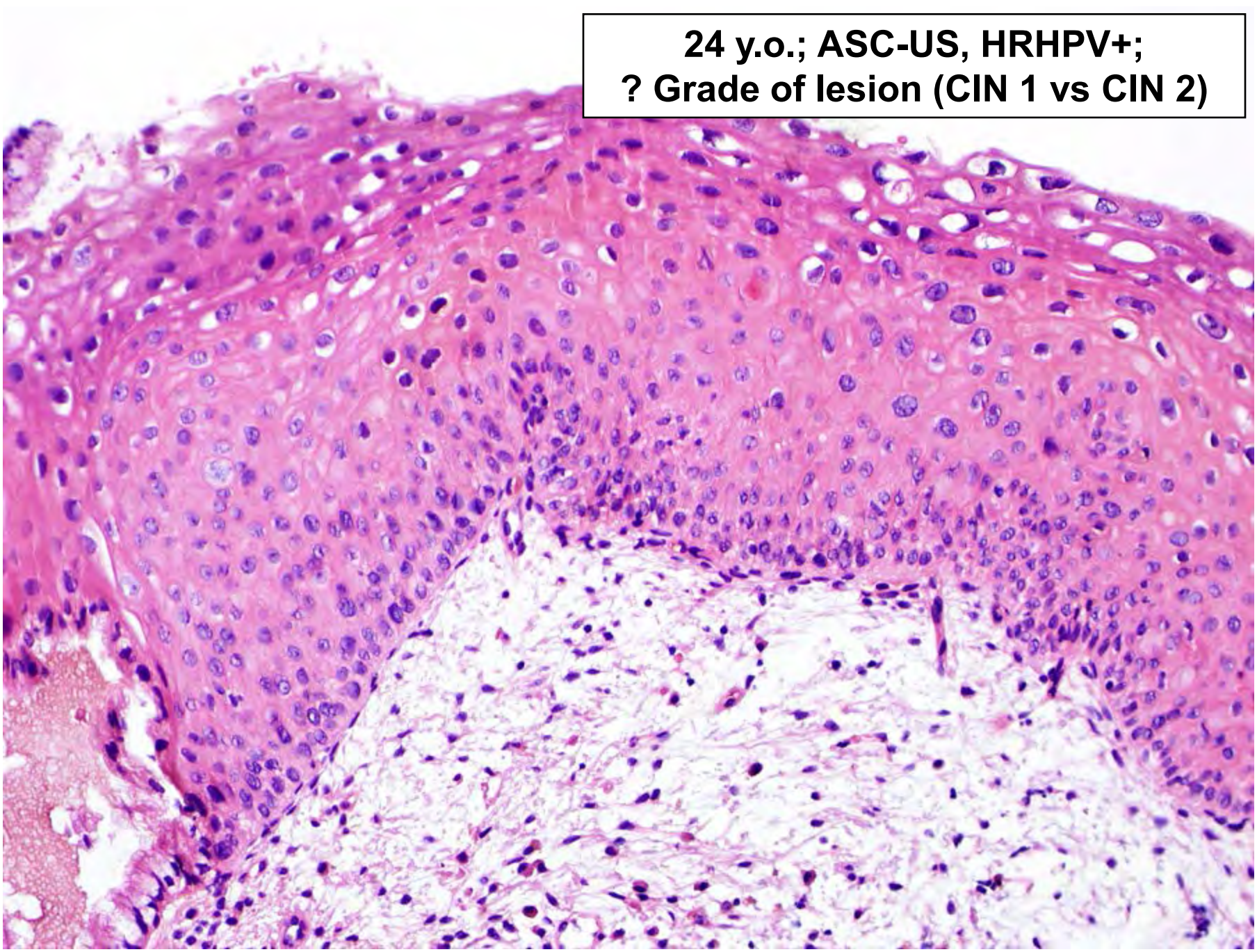


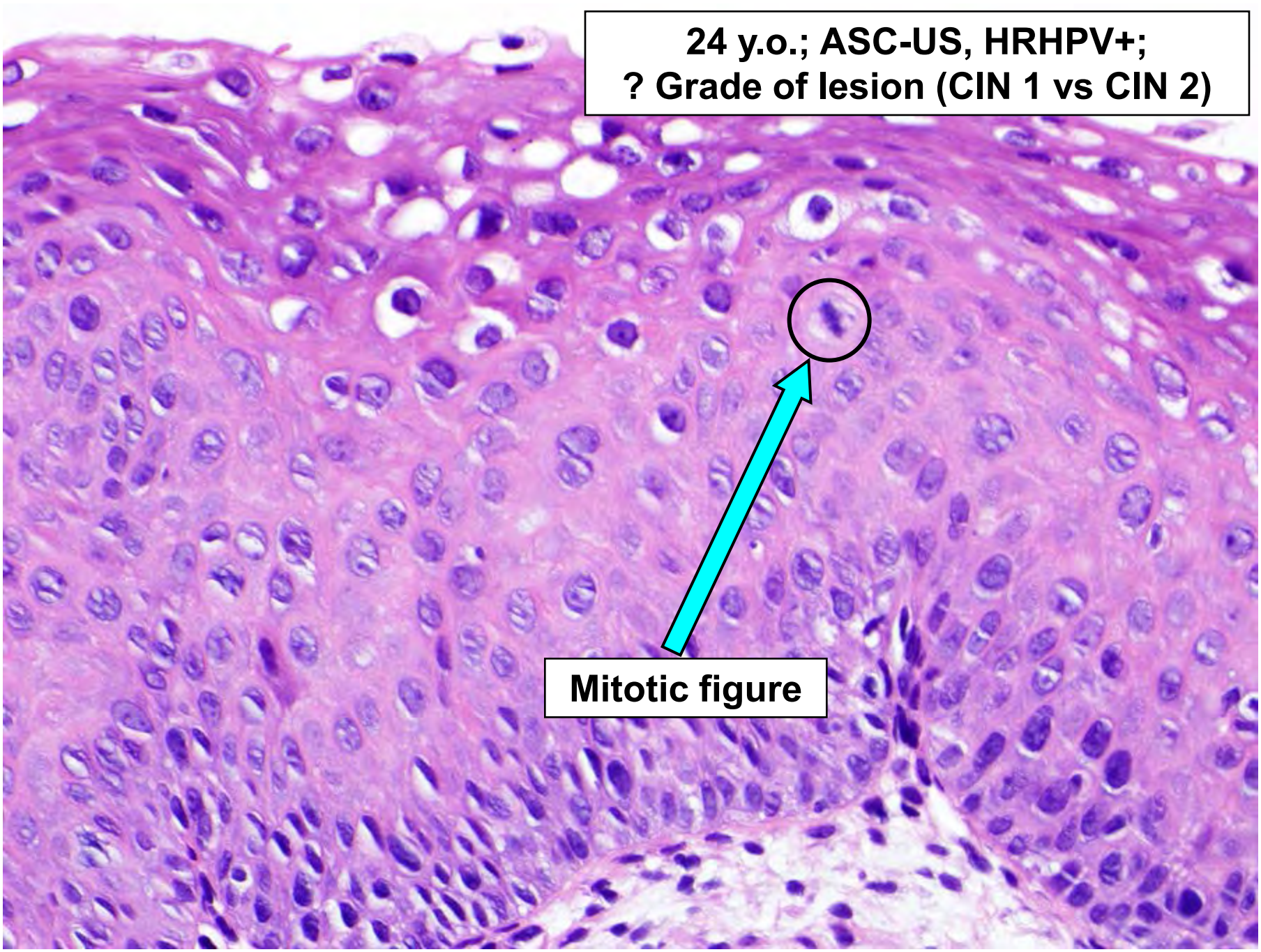
DX: Squamo-transitional metaplasia/atrophy

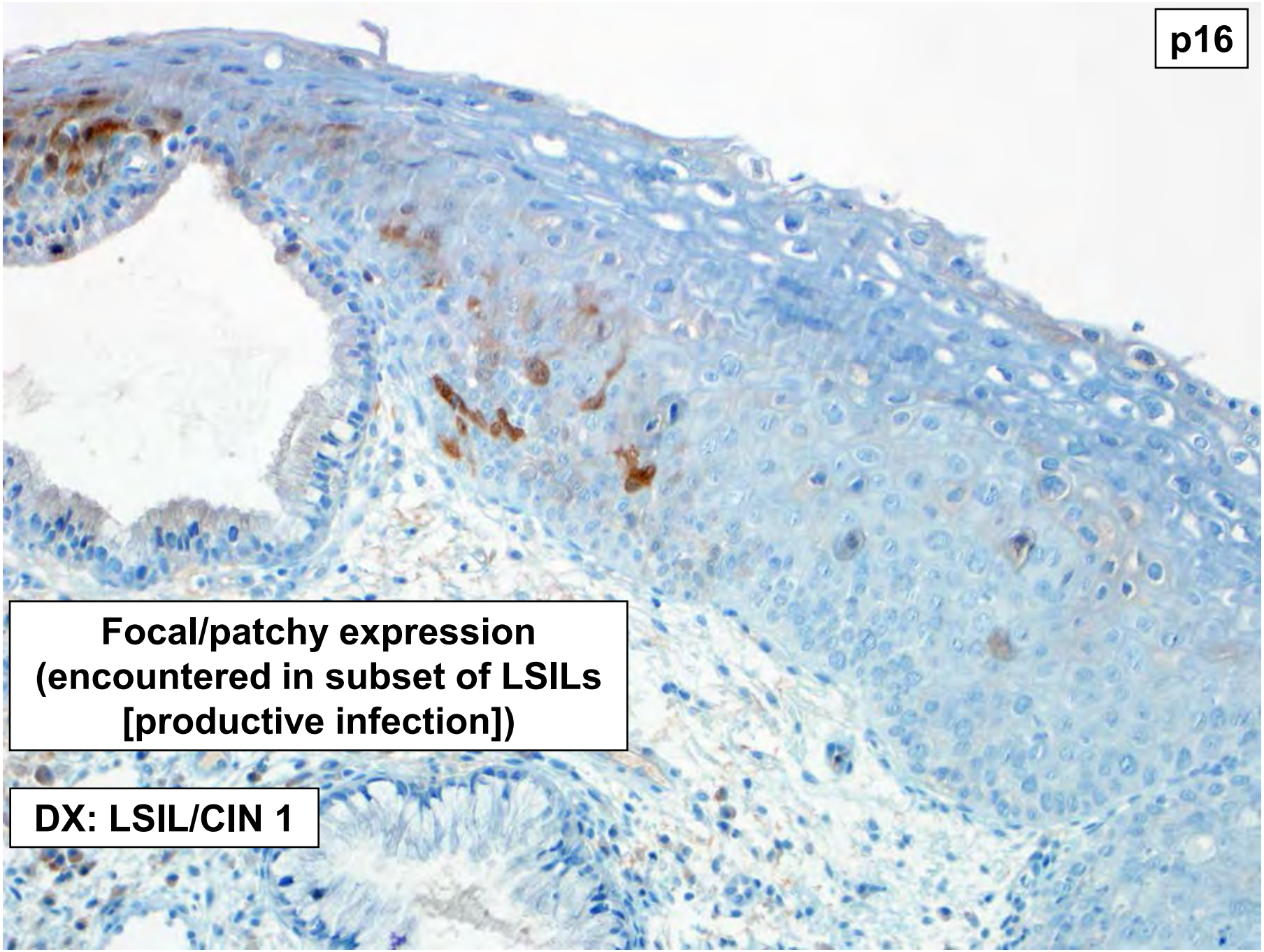
Utility of p16 in Diagnosis of Cervical Squamous Intraepithelial Lesions

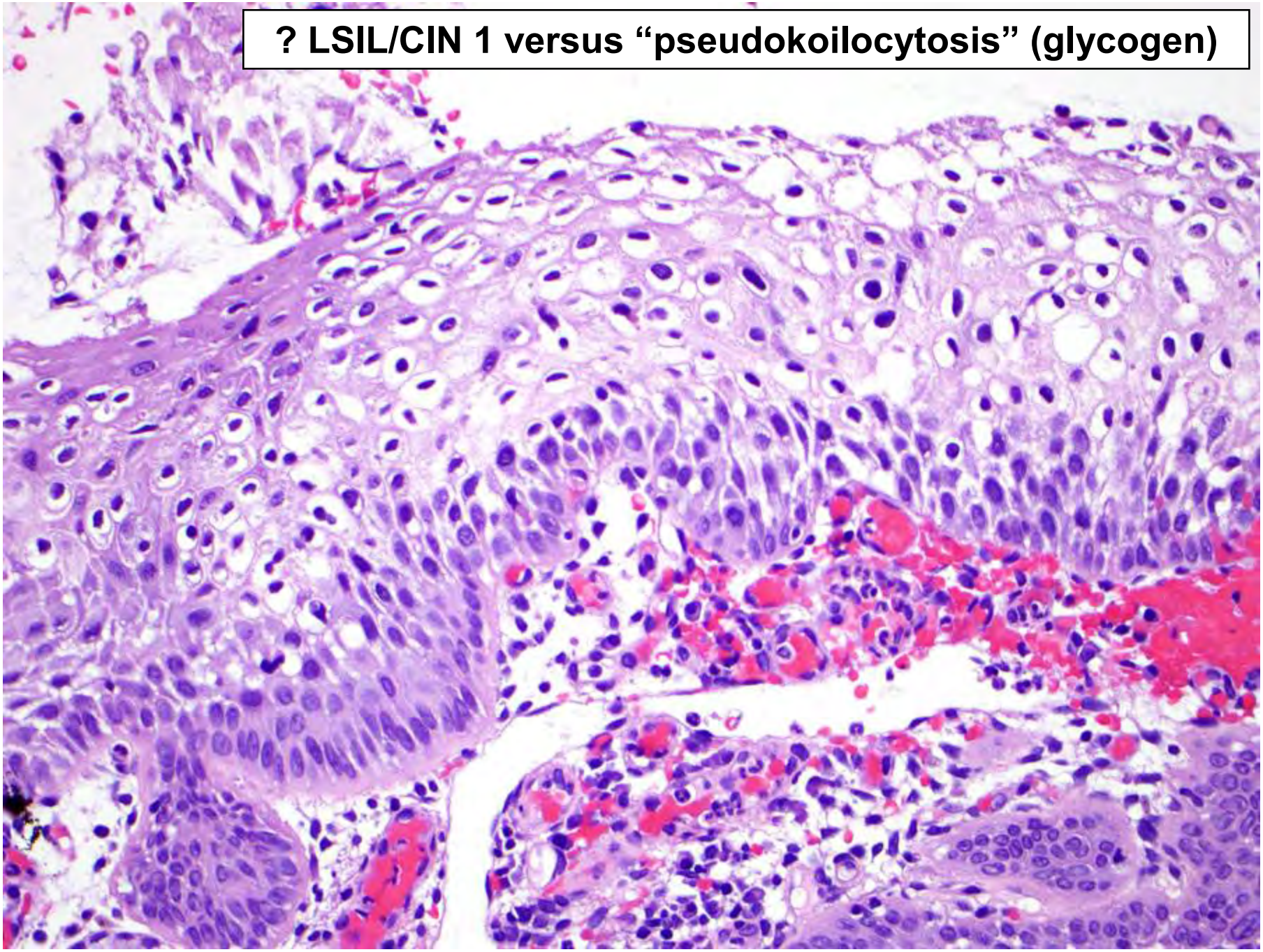
- **p16 should not be used to evaluate typical HSIL/CIN 3 and typical LSIL/CIN 1**
- **p16 is recommended when considering a diagnosis of HSIL/CIN 2 (borderline for CIN 1 versus CIN 2):**
  - **Diffuse p16 → upgrade to HSIL/CIN 2**
  - **Patchy/negative p16 → downgrade to LSIL/CIN 1**
- **Problematic issue:**
  - **Adjudicated LSIL/CIN 1: ~40-50% p16 diffuse+**
  - **Inter-observer variability in threshold for considering HSIL/CIN 2 versus LSIL/CIN 1**

CAP


24 y.o.; ASC-US, HRHPV+;
? Grade of lesion (CIN 1 vs CIN 2)


24 y.o.; ASC-US, HRHPV+;
? Grade of lesion (CIN 1 vs CIN 2)
Mitotic figure

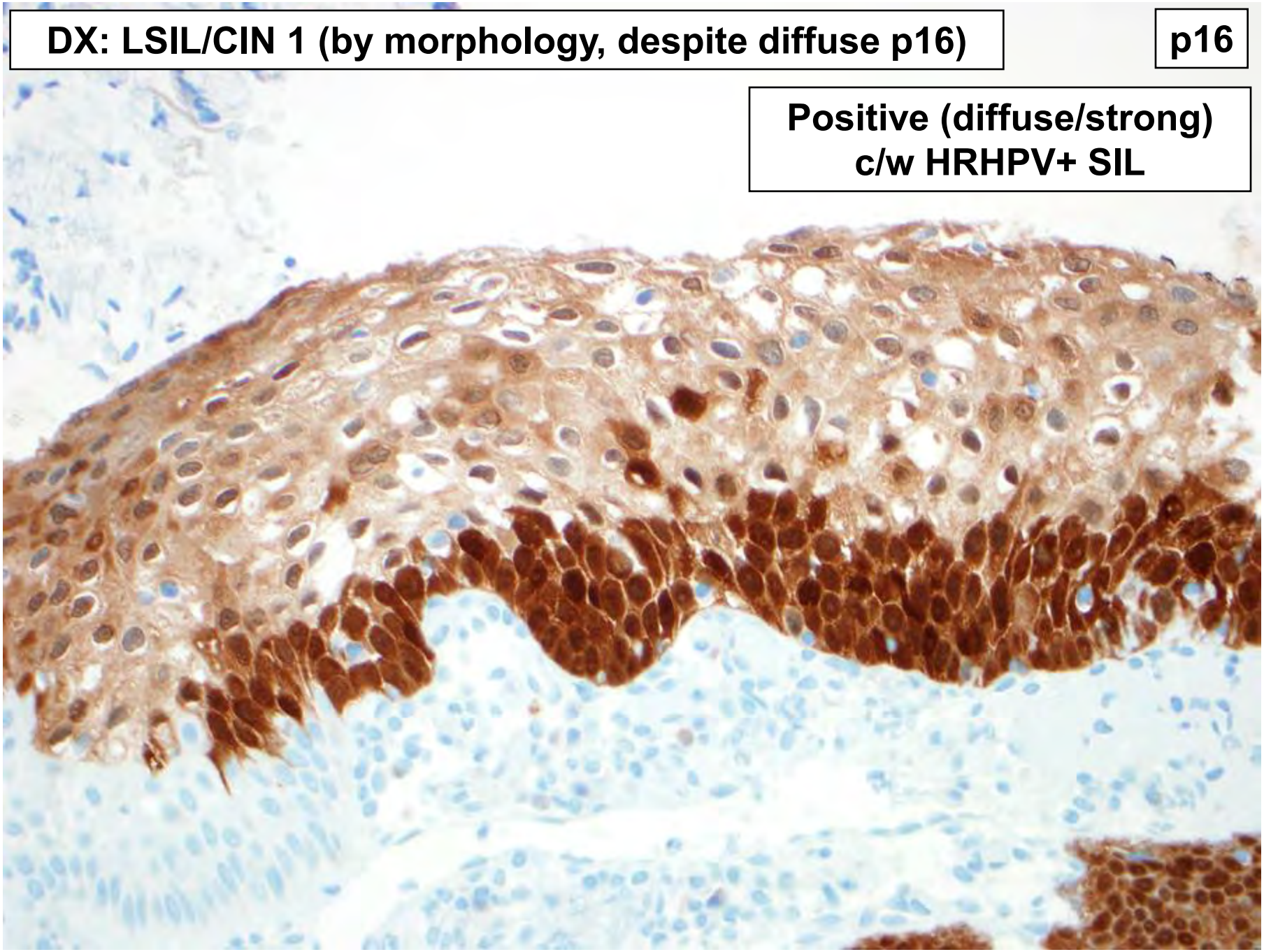

p16
Focal/patchy expression
(encountered in subset of LSILs
[productive infection])
DX: LSIL/CIN 1

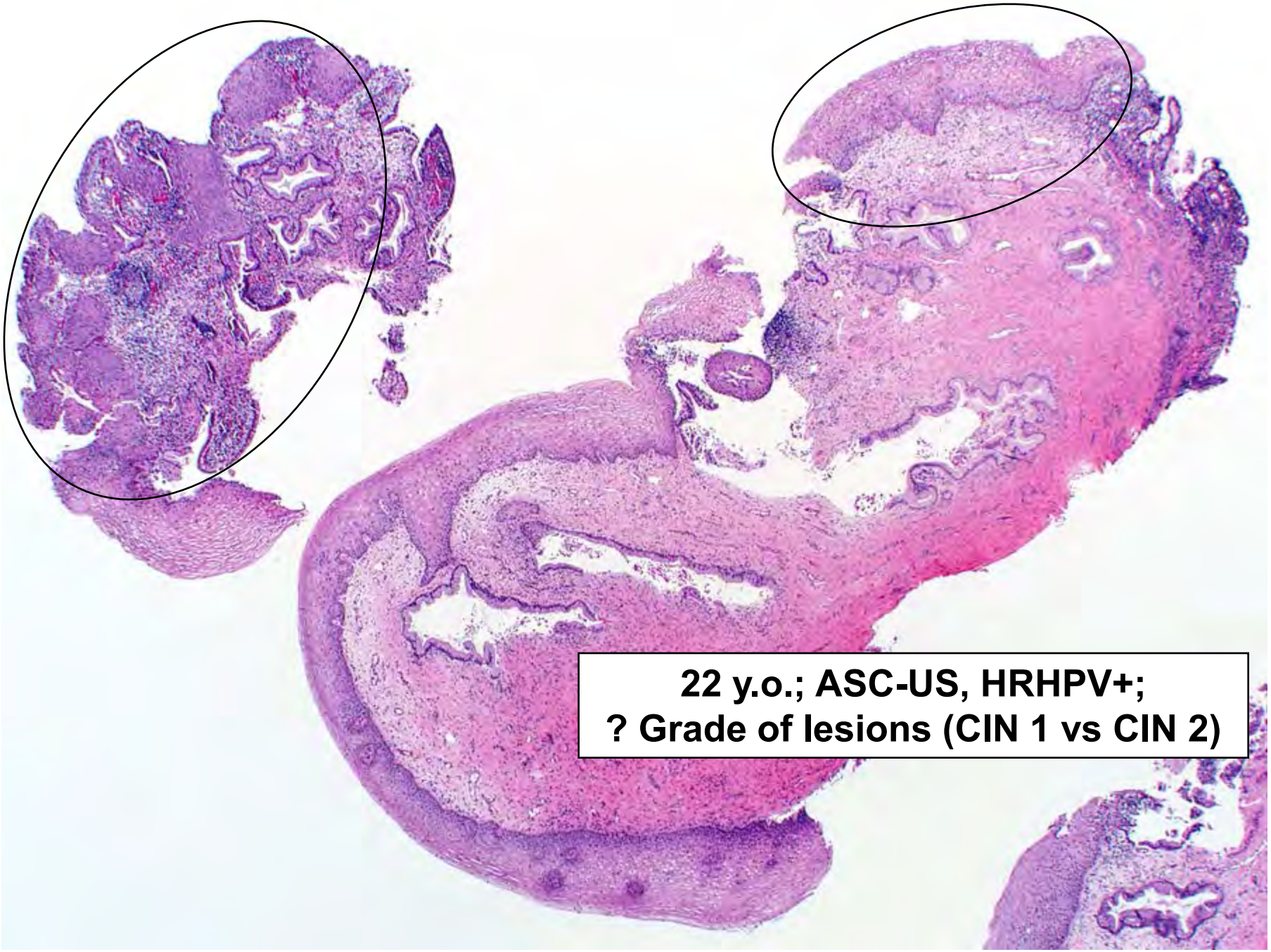

? LSIL/CIN 1 versus “pseudokoilocytosis” (glycogen)

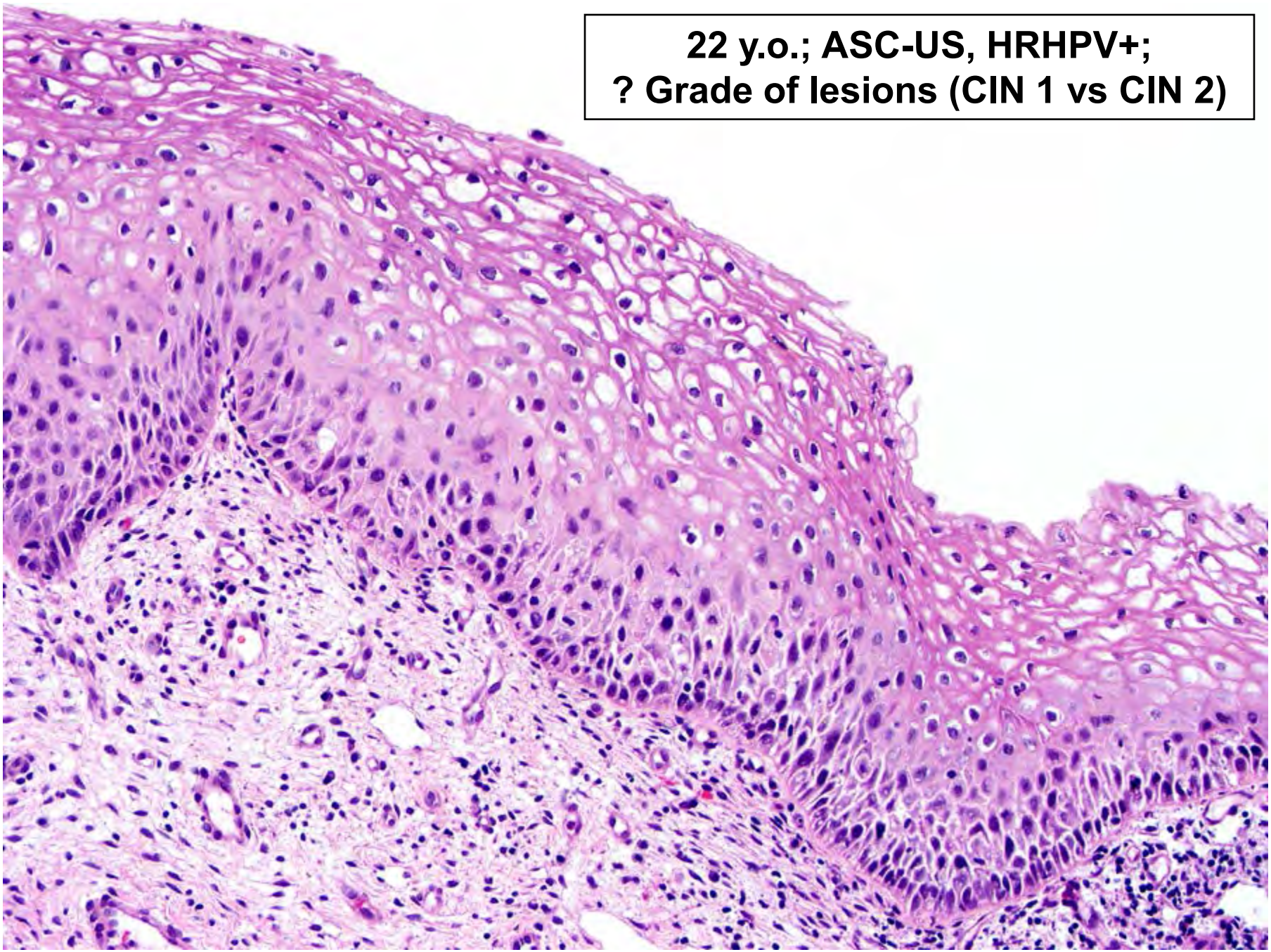
DX: LSIL/CIN 1 (by morphology, despite diffuse p16)

p16

Positive (diffuse/strong)
c/w HRHPV+ SIL



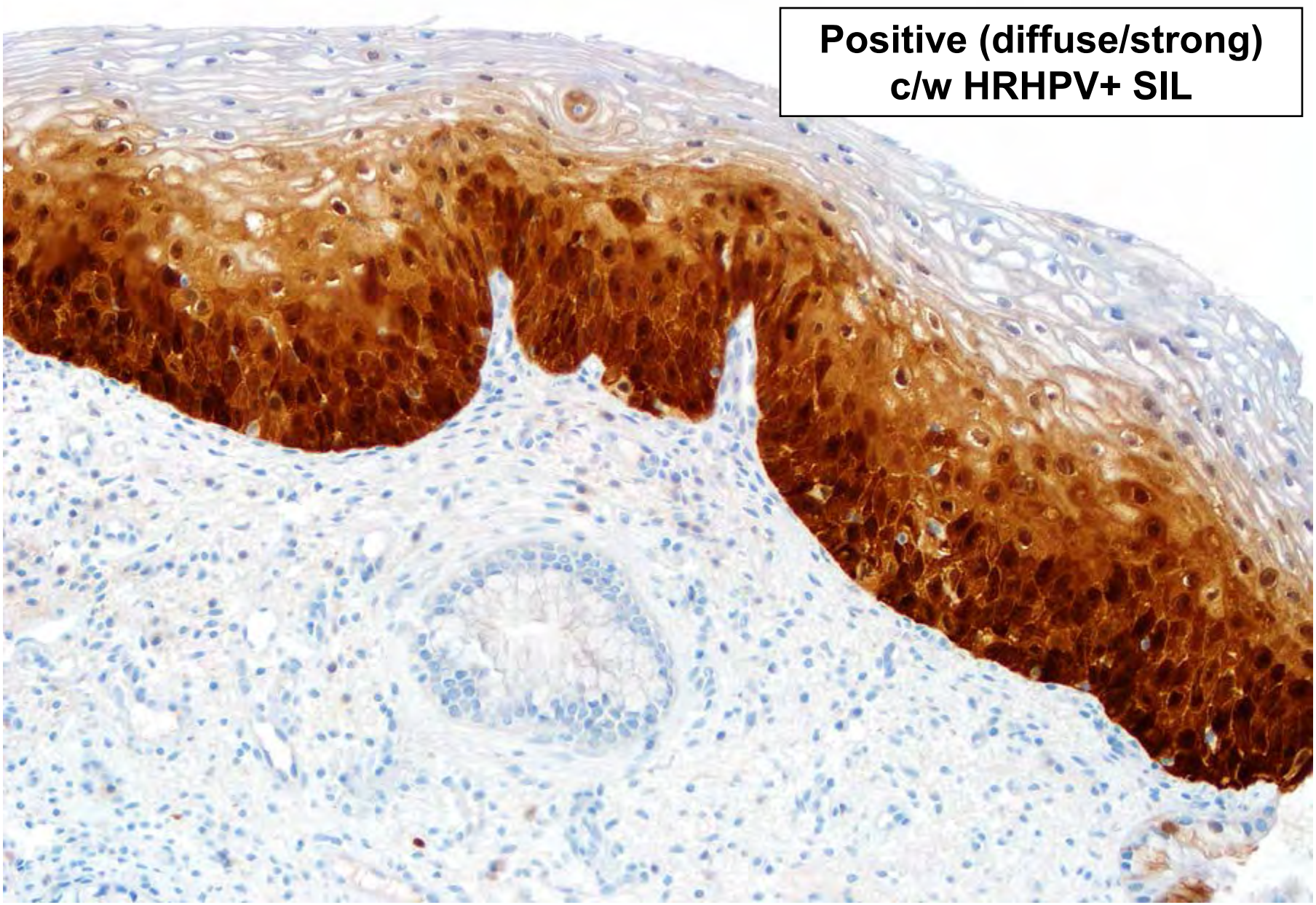

22 y.o.; ASC-US, HRHPV+;
? Grade of lesions (CIN 1 vs CIN 2)

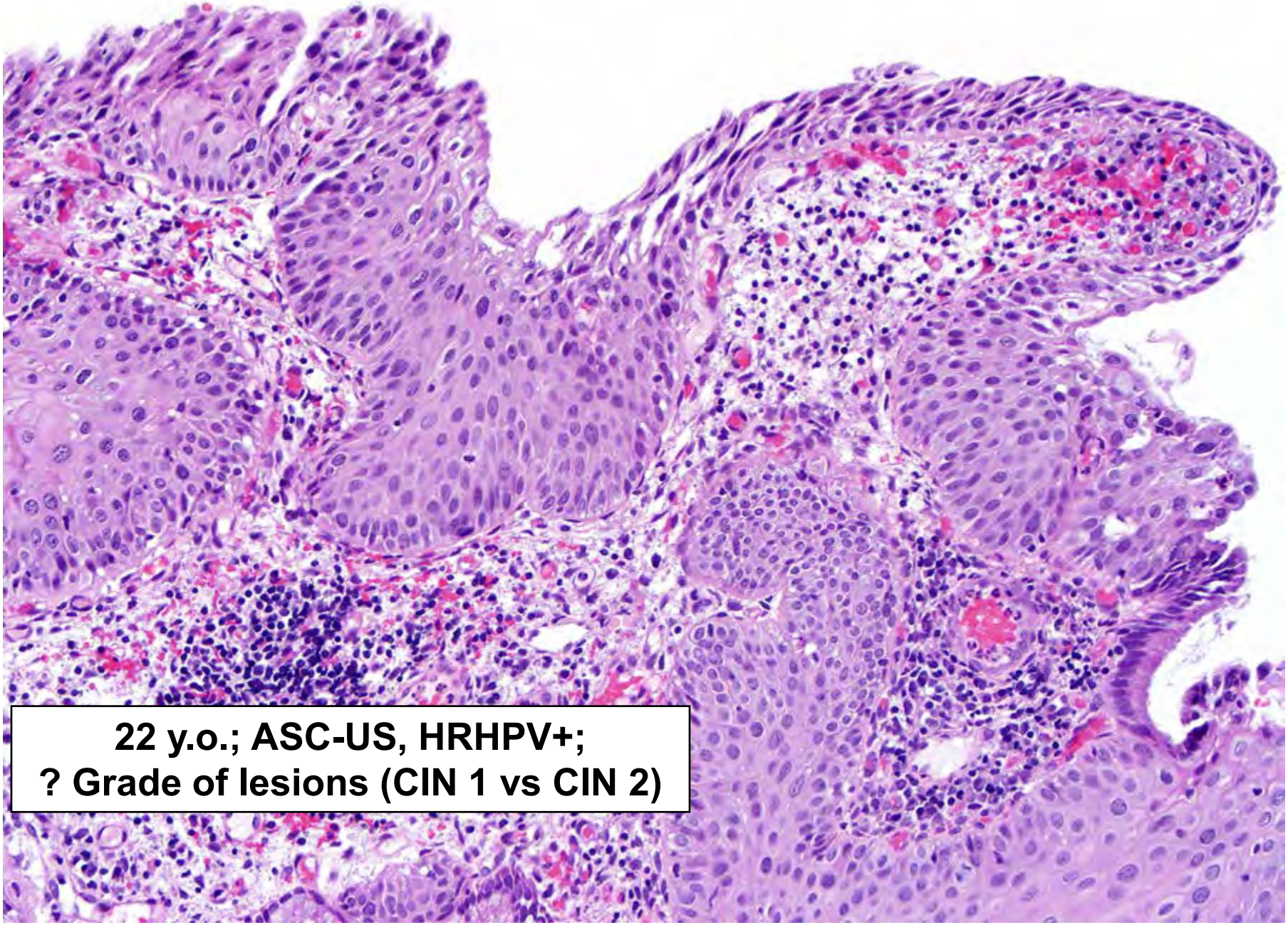

22 y.o.; ASC-US, HRHPV+;
? Grade of lesions (CIN 1 vs CIN 2)

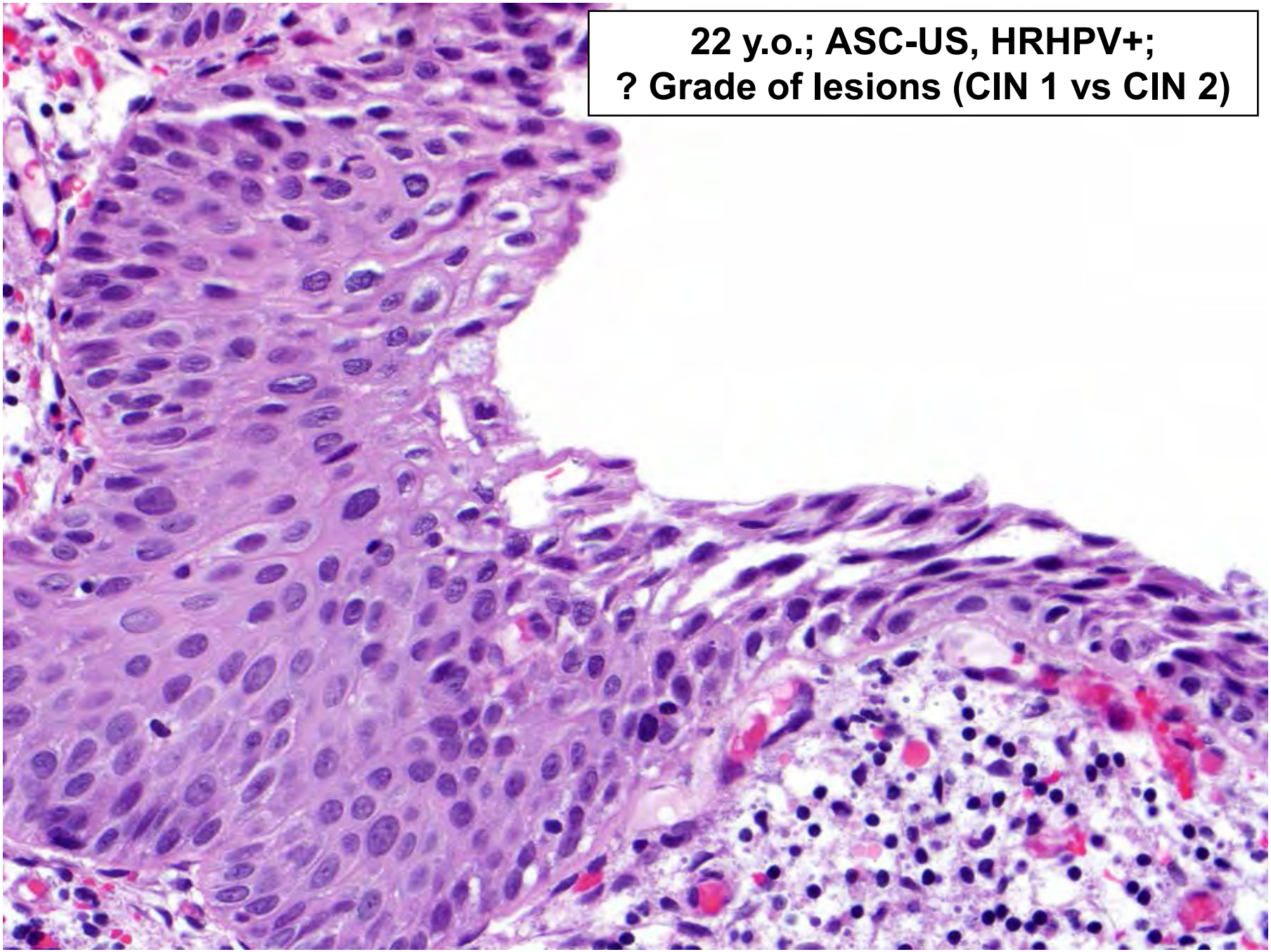
DX: LSIL/CIN 1 (by morphology, despite diffuse p16)

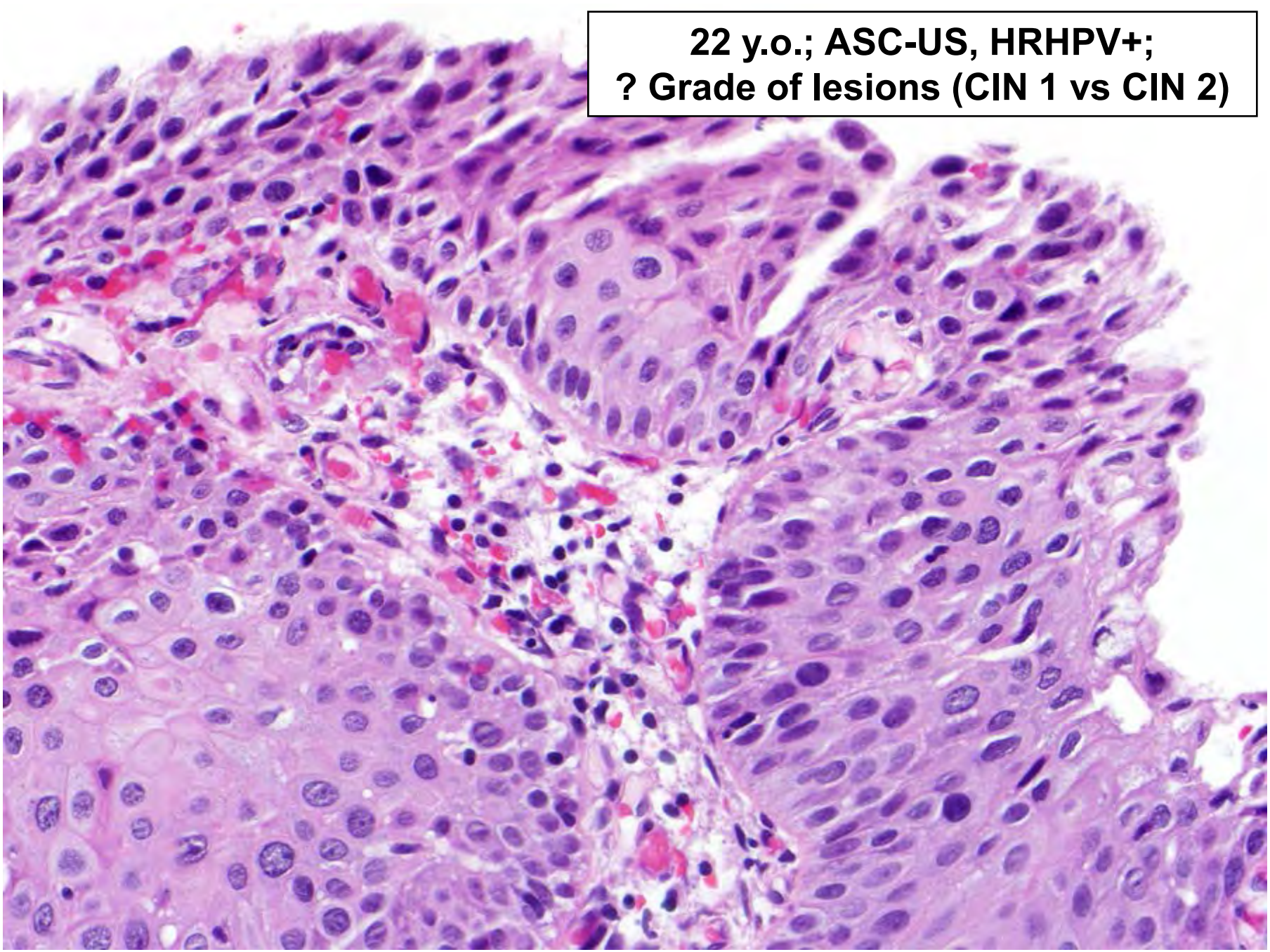
p16

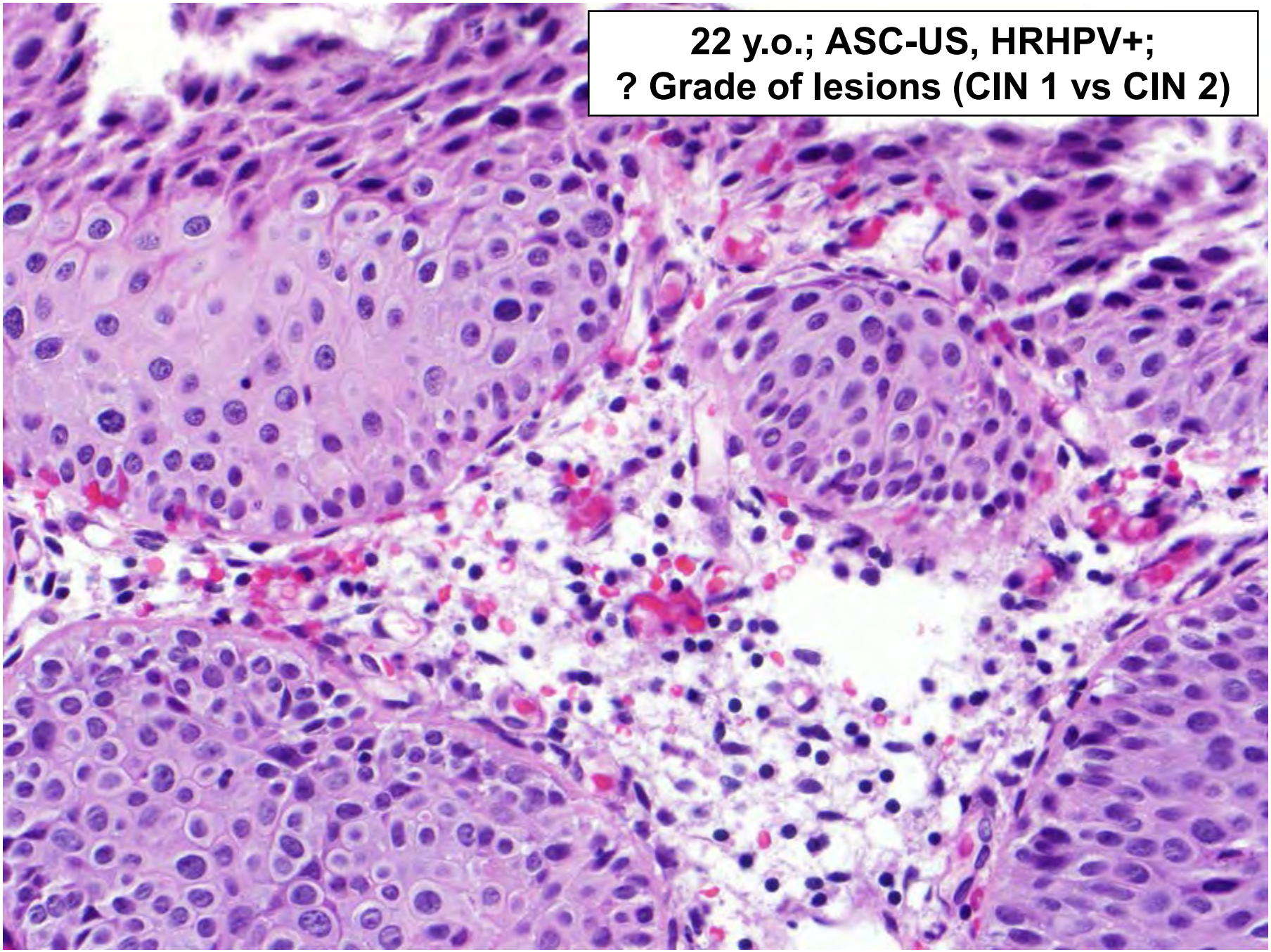
Positive (diffuse/strong)
c/w HRHPV+ SIL

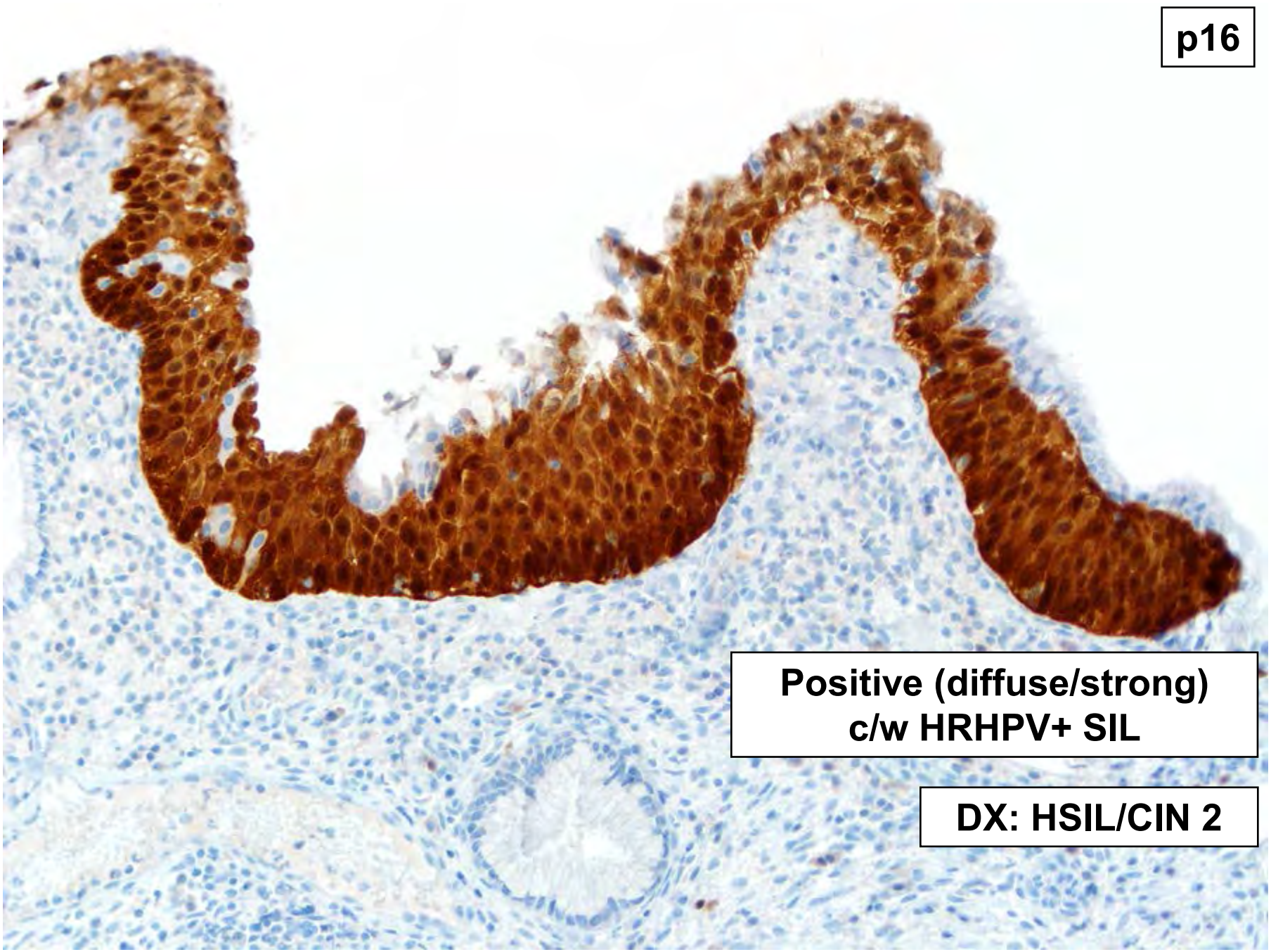


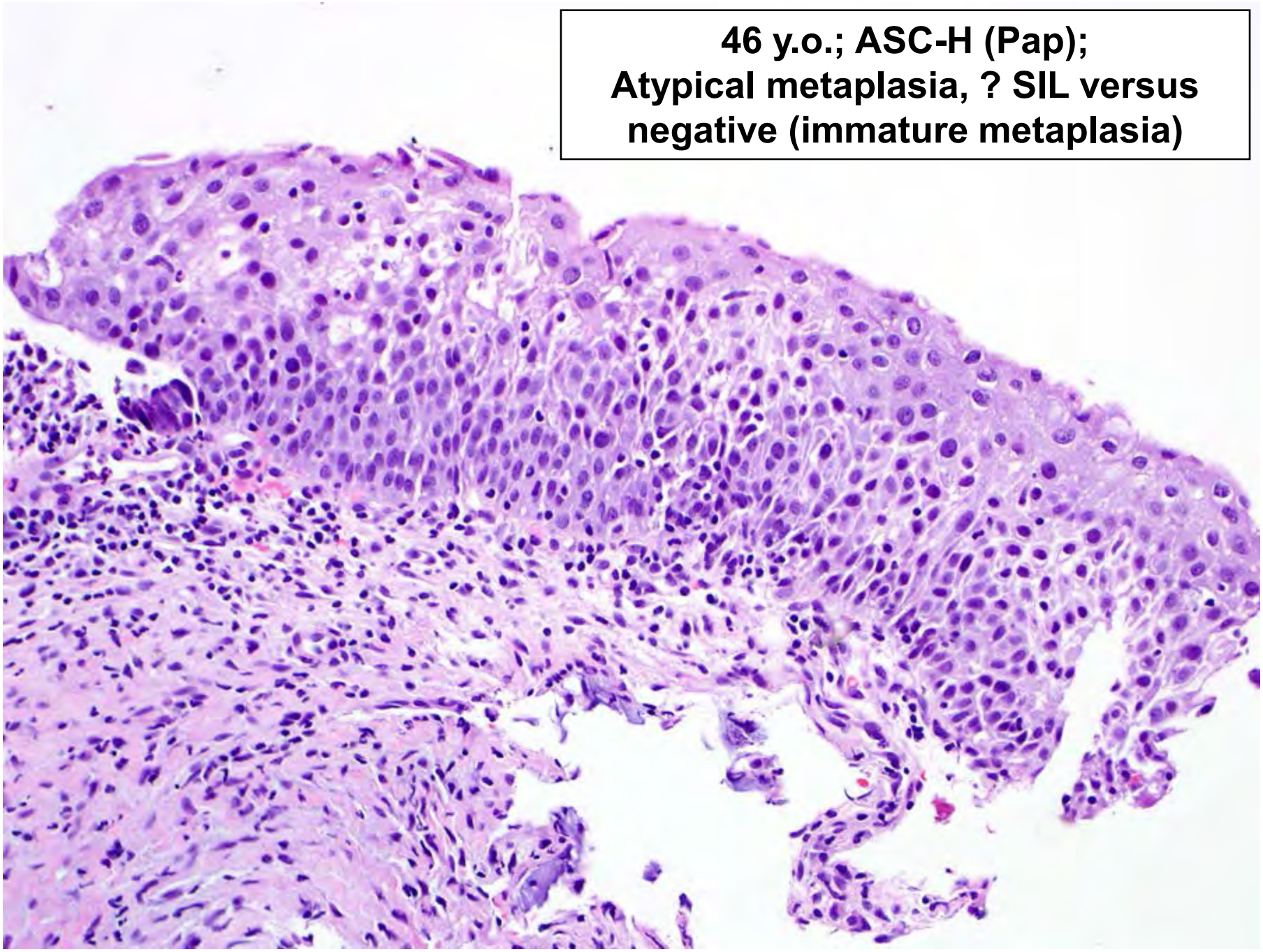

22 y.o.; ASC-US, HRHPV+;
? Grade of lesions (CIN 1 vs CIN 2)

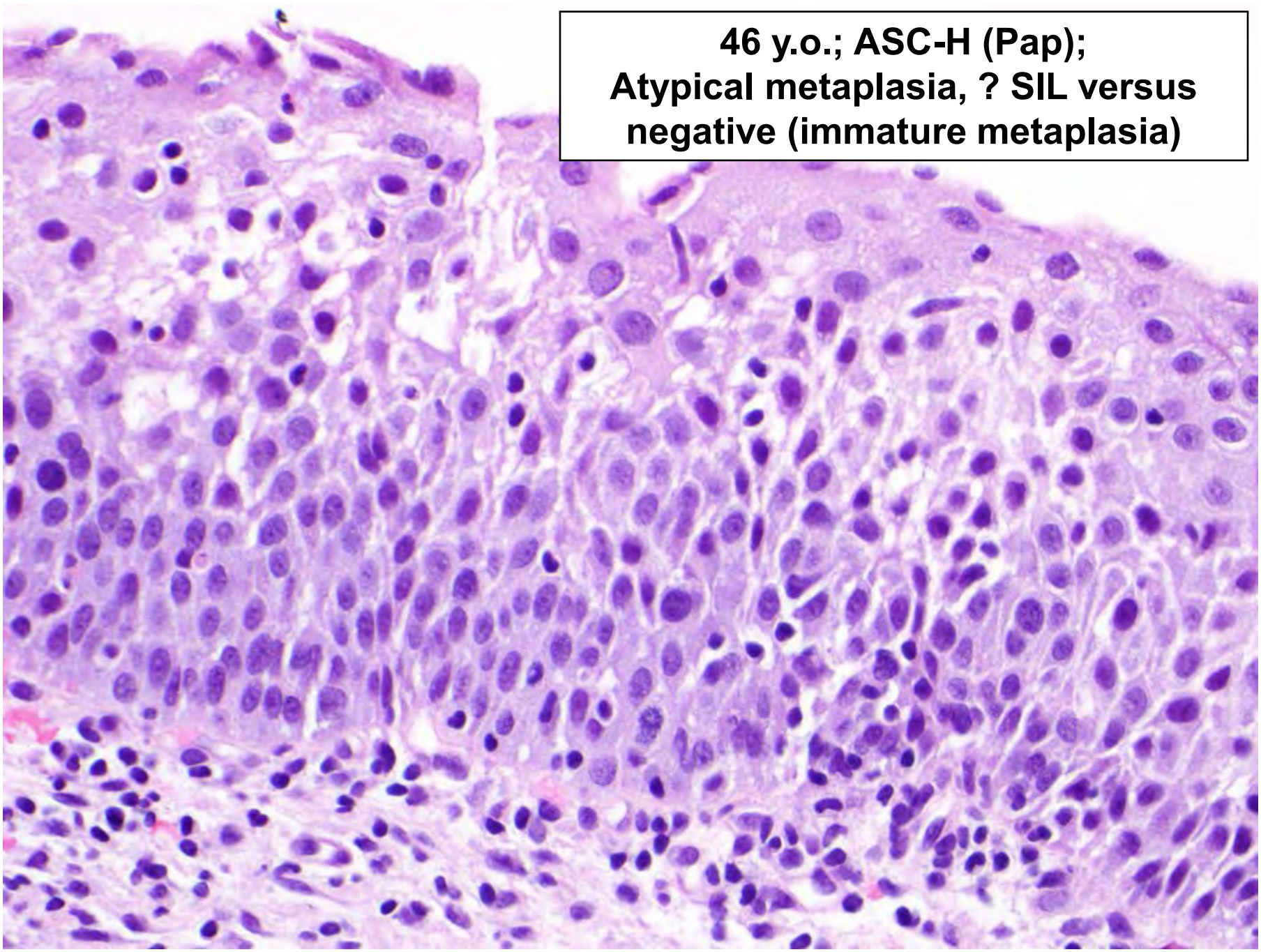

22 y.o.; ASC-US, HRHPV+;
? Grade of lesions (CIN 1 vs CIN 2)

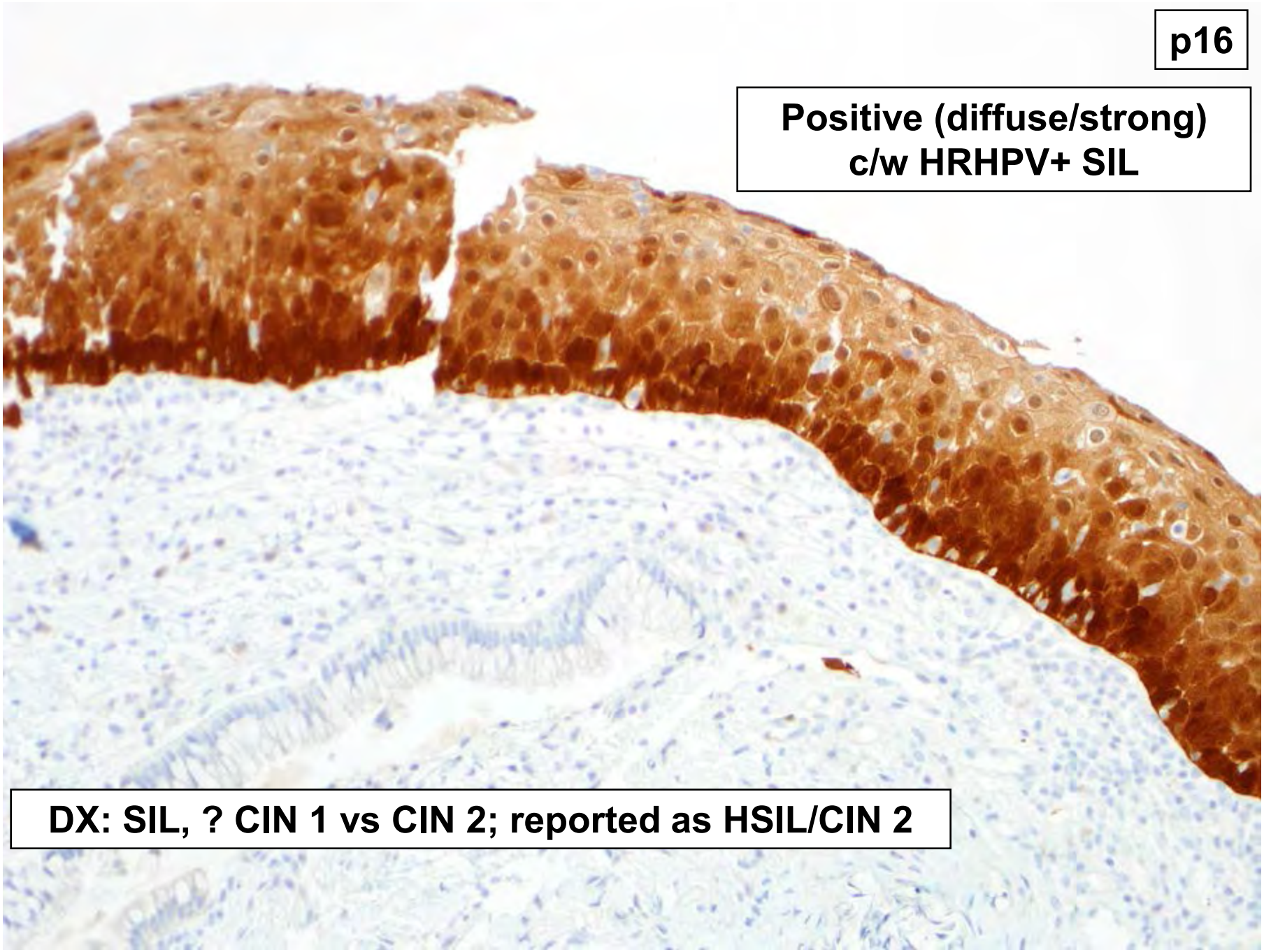

22 y.o.; ASC-US, HRHPV+;
? Grade of lesions (CIN 1 vs CIN 2)


22 y.o.; ASC-US, HRHPV+;
? Grade of lesions (CIN 1 vs CIN 2)

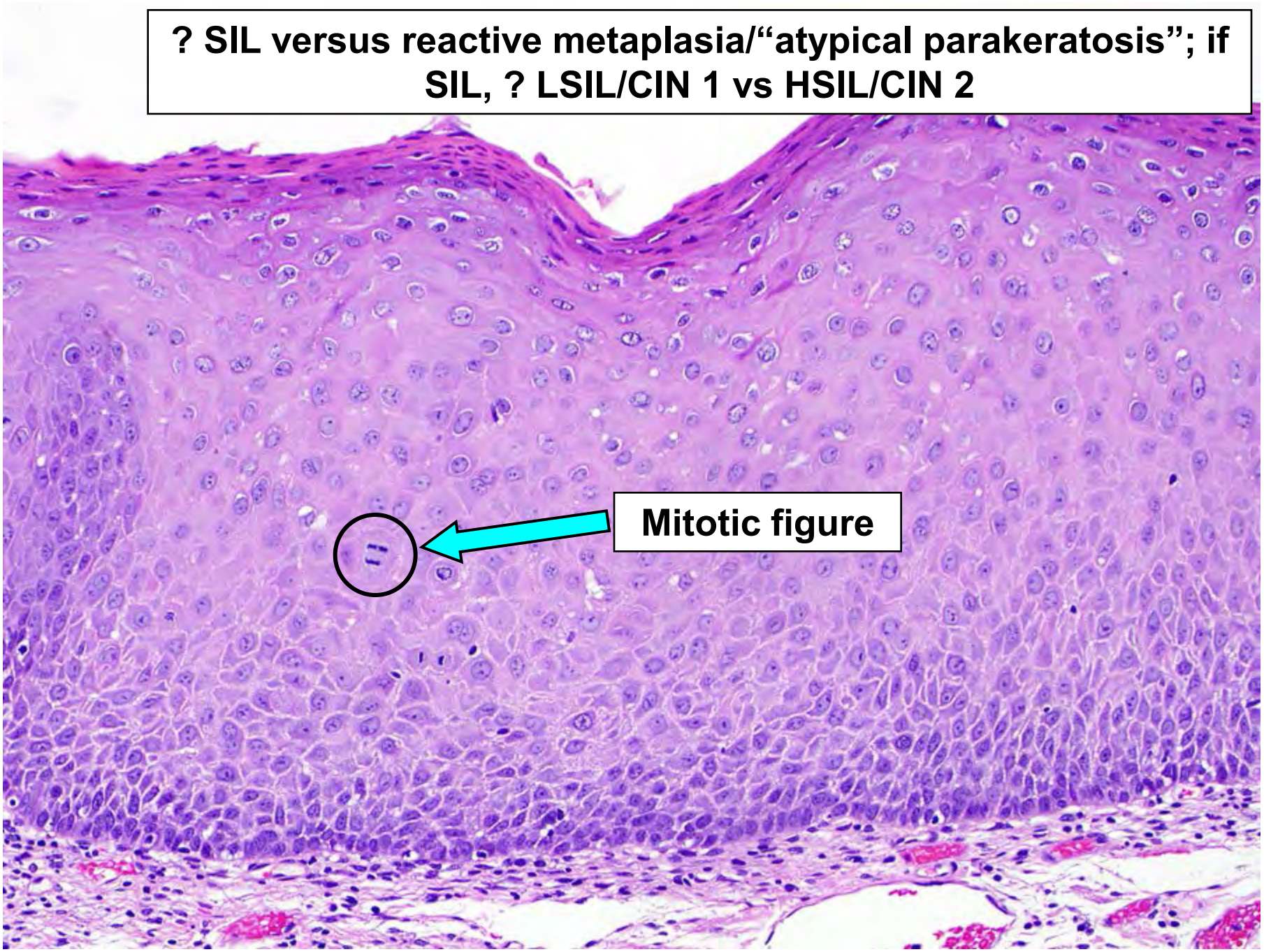

p16
Positive (diffuse/strong)
c/w HRHPV+ SIL
DX: HSIL/CIN 2


46 y.o.; ASC-H (Pap);
Atypical metaplasia, ? SIL versus
negative (immature metaplasia)


46 y.o.; ASC-H (Pap);
Atypical metaplasia, ? SIL versus
negative (immature metaplasia)


p16
Positive (diffuse/strong)
c/w HRHPV+ SIL
DX: SIL, ? CIN 1 vs CIN 2; reported as HSIL/CIN 2

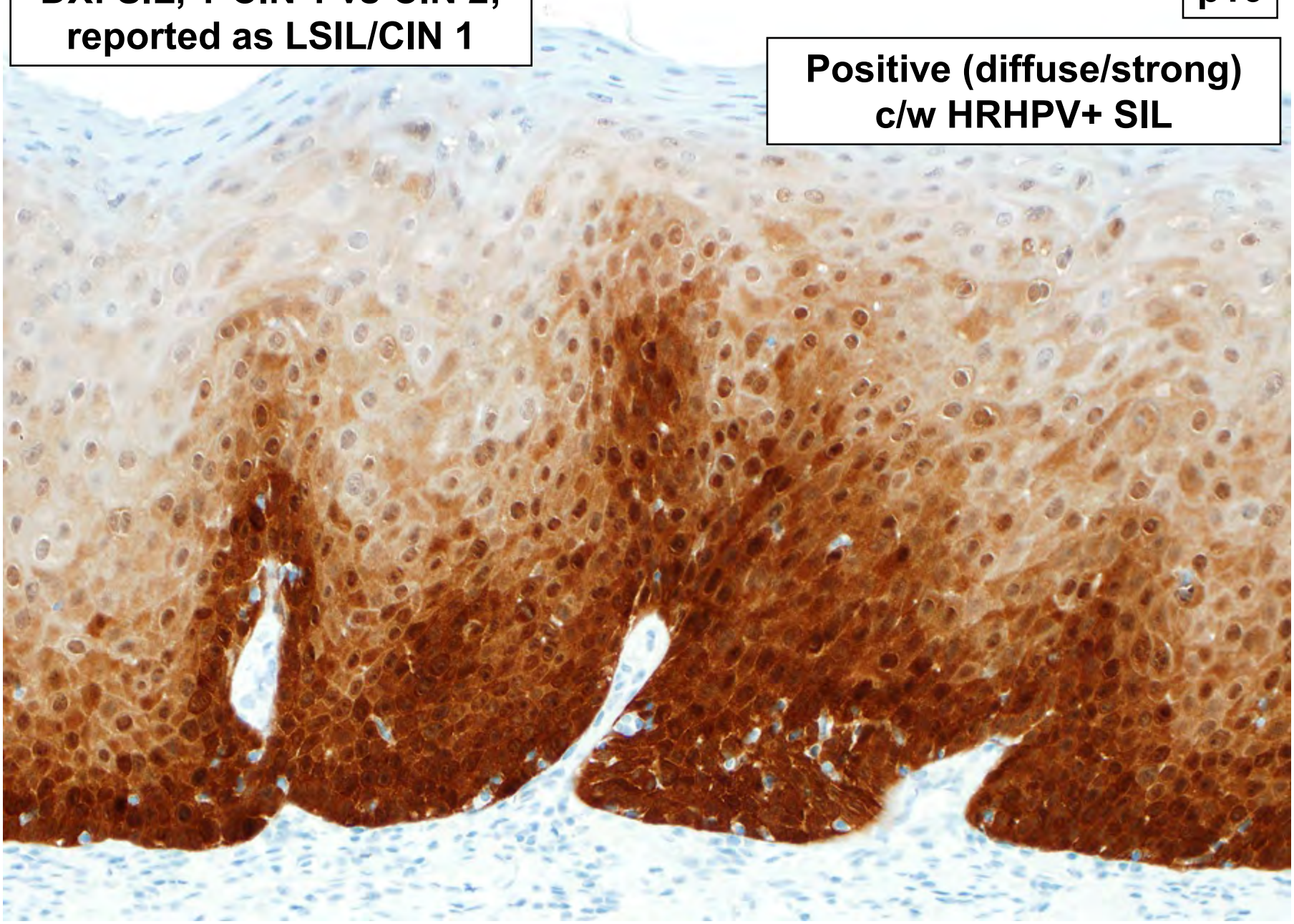
? SIL versus reactive metaplasia/“atypical parakeratosis”; if SIL, ? LSIL/CIN 1 vs HSIL/CIN 2



DX: SIL, ? CIN 1 vs CIN 2; reported as LSIL/CIN 1

p16

Positive (diffuse/strong) c/w HRHPV+ SIL



Cervical Intraepithelial Lesions: Biomarker Patterns

Coordinate expression patterns	Ki-67 ↑	Ki-67 normal/low
p16 + (diffuse/strong)	HSIL (~99%) LSIL (~40-50%)	Few/rare HSIL Rare NIL
p16 –/f+ (negative or focal/patchy)	LSIL (~50-60%) Few/rare HSIL Some reactive	NIL Reactive changes

Classification of Cervical Intraepithelial Lesions

Mild dysplasia	Moderate dysplasia	Severe dysplasia	Carcinoma in situ
CIN 1	CIN 2	CIN 3	CIS

CIN = cervical intraepithelial neoplasia

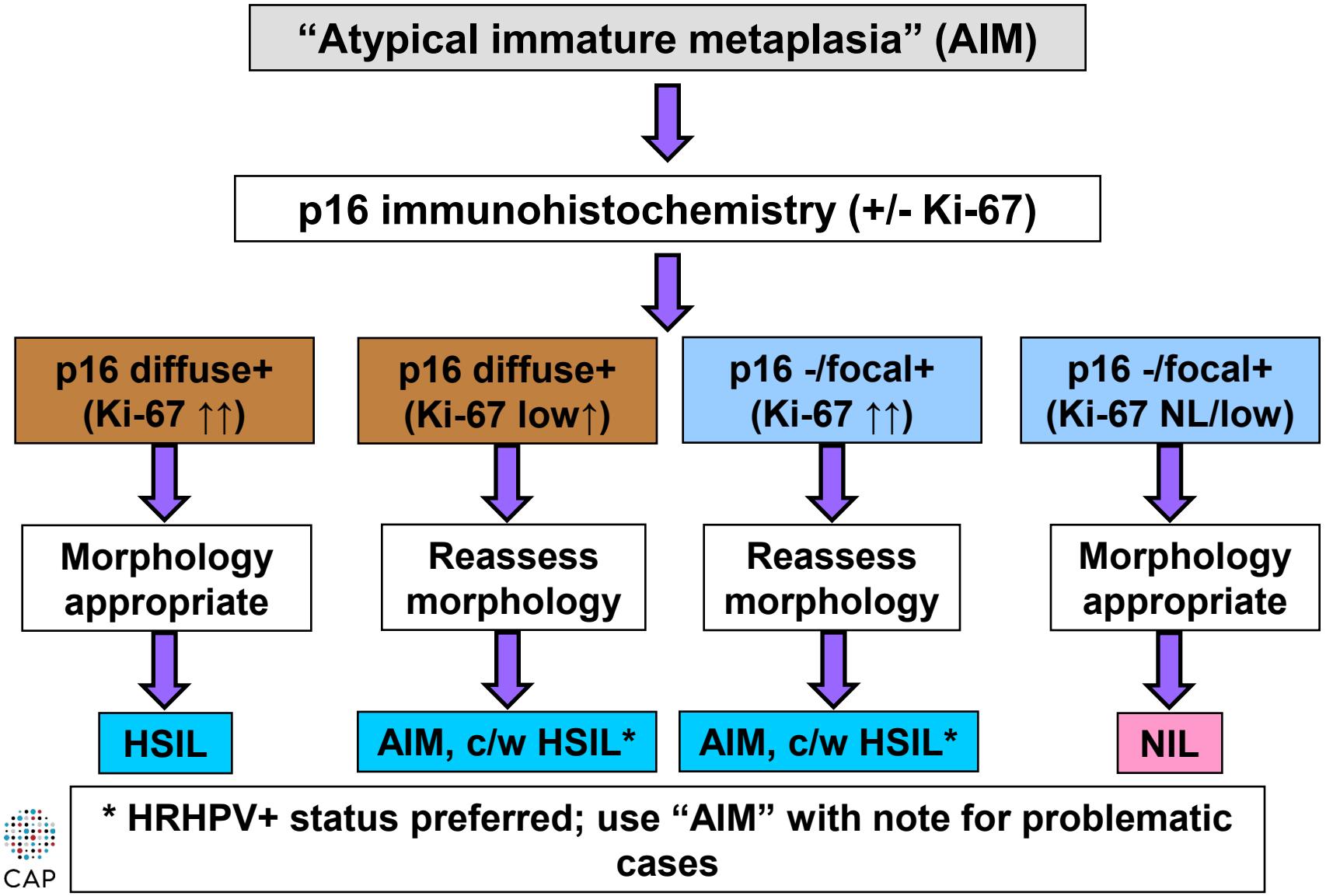
LSIL (CIN 1)	HSIL (CIN 2/CIN 3/CIS)

SIL = squamous intraepithelial lesion (low-grade and high-grade)

p16- SIL	p16+ SIL

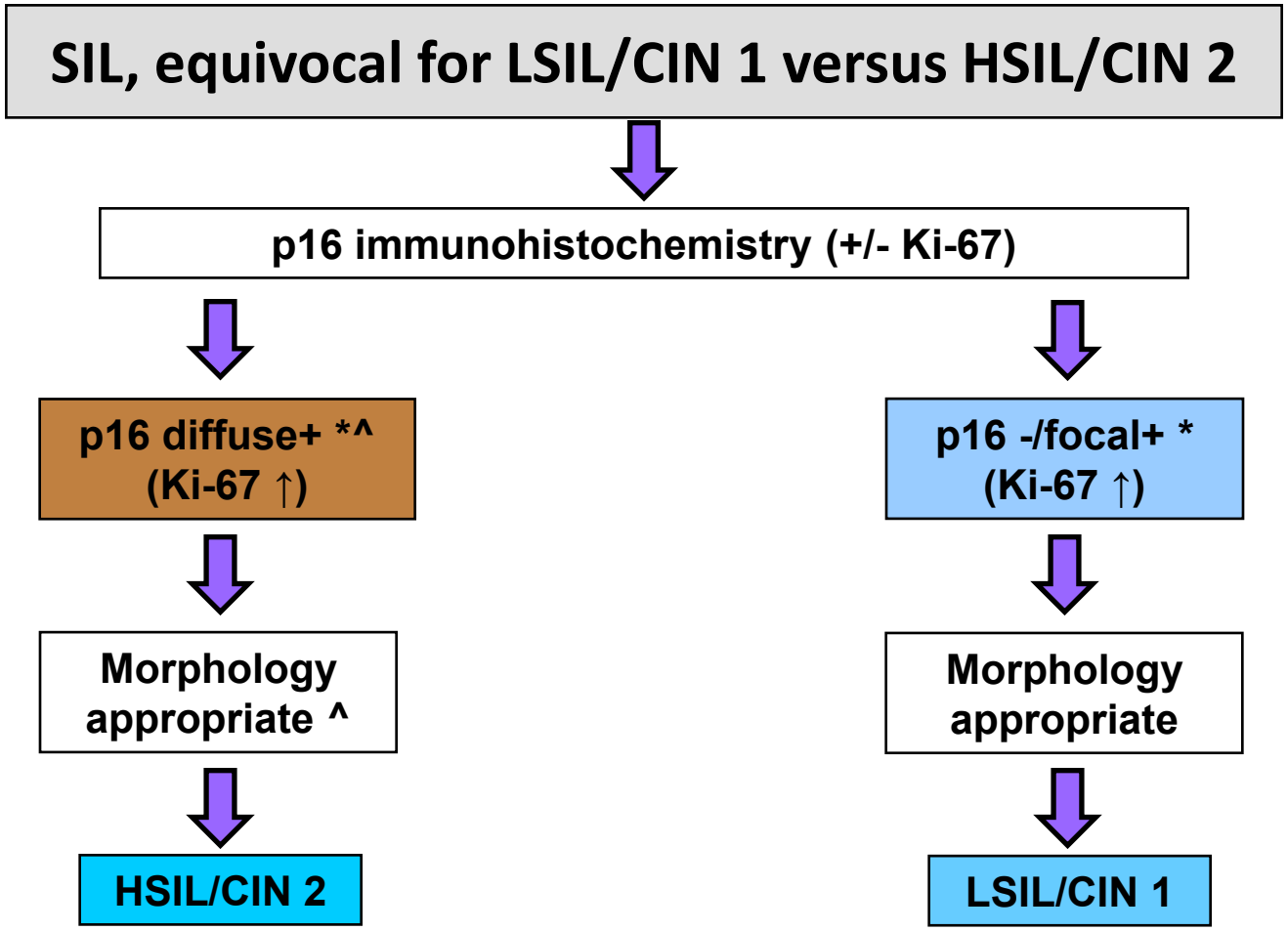
CAP

Approach to Diagnosis of Cervical Intraepithelial Lesions



CAP

Approach to Diagnosis of Cervical Intraepithelial Lesions



*both patterns encountered with HRHPV+ LSIL

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^ a pathologist may not upgrade to HSIL/CIN 2 in consultation, due to a different threshold for assessing a lesion as equivocal and since diffuse+ p16 can be c/w LSIL/CIN 1

COLLEGE of AMERICAN PATHOLOGISTS

Topic: **Applying the CAP-ASCCP LAST Project Principles in Clinical Practice: Case Examples Illustrating Biomarker Usage**

Date: June 2012

Access free archived webinar here

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Dr. Ronnett served as an expert panel member for the Intraepithelial Lesions Work Group (WG2) of the CAP-ASCCP LAST Project. She was a consultant for Merck Research Laboratories in 2012 and she gave a lecture for MTM Laboratories in 2012. In 2012, Dr. Ronnett had grants from NIH/NCI and Merck Research Laboratories. She has royalties for Blaustein's Pathology of the Female Genital Tract (Springer Verlag) (2012).

How did you diagnose the early slide of immature squamous metaplasia with positive P16?
I called this squamous metaplasia and said that, despite the extensive p16 expression, there were no morphologic features of a squamous intraepithelial lesion (either low-grade or high-grade) and that such expression has been rarely/occasionally reported in some negative cases.

Do the LSIL biopsies that show really increased Ki-67 and/or block p16-positivity - do they behave more like high grade in the long run?
There is some data to indicate that p16-positive LSIL has a greater frequency of persistence/progression than p16-negative LSIL but more analysis is needed to establish this difference. I am unaware of analysis of the significance of the degree of proliferative activity within LSILs and behavior.

In aberrant case where p16 is negative, do you think pre-analytic fixation is an issue?
Technical factors can play a role in some p16 results. A negative result should be assessed carefully to assure that there is at least some focal internal positive control to guarantee that the reaction was successful on that piece of tissue, particularly when the changes raise concern for HSIL. However, when there is patchy staining within a lesion that is morphologically consistent with or worrisome for HSIL, then that is most likely a valid yet aberrant result—that is, truly patchy and not what is expected for most HSILs. In such a case, I use the Ki-67 stain to help interpret the combined findings.

Does p16 detect patients infected with low risk strains of HPV? Could p16 negative, LGSIL and HGSIL cases be due to low risk HPV? Do you suggest doing Ki-67 in those cases? How often do you do Ki-67?
In the appropriate morphologic setting in the anogenital tract, diffuse p16 expression in a squamous lesion supports interpretation as a high-risk HPV-related lesion and thus p16 serves as a surrogate marker for high-risk HPV detection. However, nothing specific can be claimed regarding patchy or negative p16 staining and the presence or absence of any types of HPV. Low-risk HPV-related lesions, including condylomas and a small subset of LSILs, will have either patchy p16 expression or be p16-negative. High-risk HPV-related LSILs (which represent the vast majority of LSILs) can have any kind of p16 expression pattern (negative, focal/patchy, or diffuse).In the case of p16-negative or p16-patchy LSIL, a Ki-67 stain demonstrating increased proliferative activity is supportive of a diagnosis of LSIL provided the morphology is appropriate, whereas lack of increased proliferative activity supports interpretation as negative for a squamous intraepithelial lesion. However, neither stain is recommended in routine practice—LSILs and squamous atypia

borderline for LSIL versus negative should be diagnosed using H&E-based criteria (realizing that diagnostic reproducibility is suboptimal and there is a tendency to over-diagnose LSIL in routine practice). If one wants to test and adjust one's threshold for diagnosing LSIL versus negative, one can use the Ki-67 stain to adjudicate those cases (no proliferation = negative, some proliferation = LSIL). We routinely do both p16 and Ki-67 for the differential diagnosis of HSIL versus mimickers of HSIL but the current recommended approach is to do p16 alone. Ki-67 is useful for p16-aberrant cases suspected to be HSIL but these are rather uncommon in our experience.

Do you use negative and positive control with your runs?
The lab has some form of positive control for each run—either a separate tissue sample or an on-slide positive control. I do not think that we use negative controls anymore.

Is ISH for HPV useful in the difficult cases? Is there any role for HR HPV CISH in cases where p16 and Ki67 don't agree?
If one has access to HPV ISH, then trying that assay on a case with sufficient tissue remaining after doing p16 and Ki-67 can be helpful. If the result is positive, then that is supportive of interpretation as a squamous intraepithelial lesion, with grade determined by morphology. However, lack of detectable HPV by ISH does not guarantee that the tissue is truly negative for HPV. These assays have imperfect sensitivity. In our experiences, probably 10-20% of cases expected to be positive will have failure to detect HPV—these include some cases that must have high-risk HPV based on their morphology (e.g., some adenocarcinomas with AIS, some squamous cell carcinomas) and which have been proven to contain high-risk HPV by PCR when we investigated them. For this reason, we never report an HPV ISH result as negative for HPV—rather, we use the phrase "no detectable HPV", particularly for those cases that are likely a failure to detect.

If p16, KI 67 are not conclusive for High grade lesion, but HPV testing is (+), will your clinician proceed with Cone?
Management is dependent on multiple factors, including prior history (Pap and biopsy results), current diagnostic interpretation, patient age, etc. I do not know how all clinicians will manage a biopsy diagnosis of "atypical immature metaplasia; HSIL cannot be excluded due to inconclusive or conflicting or aberrant immunohistochemical results. I try to favor one process or another based on the combined findings so as to guide the clinicians as much as I can. Therefore, if most but not all factors lean toward a diagnosis of HSIL then I will favor that diagnosis. For example, when morphology and Ki-67 favor HSIL in a patient who is HRHPV+ but p16 is aberrant patchy+ I would favor HSIL so the clinician is encouraged to act on that favored diagnosis. If both stains do not support HSIL then one has to consider or conclude that the lesion is a mimicker of HSIL. I don't think that simply being HRHPV+ is enough to warrant a cone biopsy but in certain situations, equivocal biopsy results plus HRHPV+ status might lead to such management when the patient age and fertility considerations are appropriate and there is either persistent atypia by Pap and/or biopsy and/or the colposcopic evaluation is inadequate.

Basal patchy p16 pattern vs. surface pathy p16. Is there and significance? I.e., does it suggest a dx?
I am unaware of any specific significance of the location of the patchy pattern. Either pattern would be considered patchy (non-diffuse) and, therefore, not a significant/positive staining pattern.

In several of your examples, you have chosen to not to use Ki67 because the P16 was negative and your morphologic assessment was negative; however, by doing this you have selected against finding those problematic cases that may be p16 negative and Ki67 positive. Why not do both stains on all problematic cases?
We routinely do both stains on problematic cases. I actually removed all of the Ki-67 stains from the lecture in the interest of time and to focus on the specific recommendations adopted by the LAST consensus conference—that is, to use p16 alone without Ki-67. I chose to illustrate Ki-67 only for those cases in which the p16 result was aberrant, to show how I made a final interpretation based on the combined findings. There are some cases for which the Ki-67 result is somewhat lower than expected for typical HSIL (but not "negative"/no increased proliferation) but the p16 is positive (diffuse). In those examples I use the morphology plus diffuse p16 result to diagnose HSIL despite the lower than expected Ki-67 result. The very few p16-negative/patchy cases with increased Ki-67

I showed as examples of p16-aberrant HSIL represent the only ones I have encountered despite routinely doing both p16 and Ki-67 on virtually all problematic cases, so I think this situation is (fortunately) rare enough that p16 alone actually catches nearly all cases. Ki-67 can be added when the morphology and p16 appear discordant.

How often will you see p16+ lesions with HPV negativity? Are some cancers/HSILS not related to HPV, or do you think there had been a previous HPV infection in all HSILS cases?
It depends what you mean by HPV negativity or positivity. By testing a cytology specimen from a patient with a commercial test or by PCR analysis of the tissue specimen? Biopsy tissues do not get tested for the presence of HPV (by HPV ISH or PCR) in routine practice but we usually do know whether the patient had a liquid-based HPV test. Data from large studies indicate that adjudicated HSIL (tissue diagnosis) has a very high frequency of diffuse p16 expression (~99%) and HRHPV positivity by PCR (over 90%) and that virtually all cervical squamous cell carcinomas are HRHPV+.

What is the long term clinical follow up for the diffusely + p16 lesions that look low grade? How many will progress to HSILS?
There is limited data in the literature to indicate that there is an increased frequency of persistence/progression for p16+ LSIL compared with p16-/patchy LSIL but more analysis is needed.

Do you ever diagnose CIN2 without the p16 immunostain?
I certainly did before the LAST recommendations were made. However, now the recommendation is that p16 be used to adjudicate all cases for which a diagnosis of CIN 2 is being considered.

Could you comment on the utility of p16 and ki67 for hpv-related lesions of the vulva (condyloma, vin1, 2 & 3)? Could P16 apply to anal biopsy or vulva or vagina bx? Could you comment on the use of routine HPV sub typing by ISH (high and low risk) in anal squamous intraepithelial lesions?
I use p16 and Ki-67 for vulvar lesions, and other anogenital sites, in the exact same way as I do for cervical lesions. We do not use HPV ISH routinely for any of the lower anogenital sites but do use it selectively in certain situations (for example, we use HRHPV ISH for certain tumor situations to supplement p16 and use type-specific HPV ISH probes [HPV 6/11 and HPV WS] for diagnosing condylomas).

Some of the cases you said were LSIL but had diffuse p16 staining showed diffuse staining but only in the bottom third to half of the epithelium. Is this really "diffuse" staining?
As I described in some of the examples, diffuse p16 expression is defined as diffuse staining in at least the lower half to two-thirds of the lesional epithelium and does not require the staining to be completely full-thickness. Thus, diffuse refers more to the horizontal extent than the vertical extent of staining (it can be full-thickness but this is not mandatory).

Is p16 only cytoplasmic?
p16 staining is generally diffuse throughout the cells, including cytoplasmic and nuclear expression.

Please pass on to Dr. Ronnett that this was a helpful, very practical review of issues that we encounter every day. Thank you!!!
I am glad this was helpful.